DENGUE AND CHIKUNGUNYA

The tiger mosquito, *Aedes albopictus*, a vector of chikungunya and dengue viruses, and its cousin *Aedes aegypti*, the principle vector of dengue fever, have both evolved life cycles closely linked to human habitation. Females like to lay eggs in domestic water and sanitation systems, and any collected water such as in used tires and discarded water containers.
Dengue is the fastest growing global health problem caused by a mosquito-borne disease.

Estimates range from 50 to 100 million cases a year – even 400 million might be realistic.

1 to 2.5 billion people live in dengue endemic areas.

Insecticide-treated materials (ITMs) can have a dramatic impact on vector populations.

Dengue is endemic throughout tropical and sub-tropical regions all over the world.

Integrated vector management (IVM) and integrated resistance management (IRM) strategies must have high priority.

500,000 severe cases of dengue hemorrhagic fever (DHF) occur each year.

Aedes mosquitoes bite during the day.
**Research Article (Example)**

**Key Facts About Dengue**

- Dengue is transmitted by *Aedes* mosquitoes: *Aedes aegypti* and *Aedes albopictus* (Asian tiger mosquito).
- The Asian tiger mosquito (*Aedes albopictus*) is responsible for recent outbreaks of dengue in France and Croatia (2010).
- *Aedes* mosquitoes have adapted their life cycles to conditions of human domestication.
- Dengue fever is caused by four different, but closely related flavivirus species.
- Fatal forms of the disease are dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).
- Possible vaccines for dengue must neutralize all four virus types.
- Dengue is spreading to previously uninfected areas.

Dengue is endemic throughout tropical and sub-tropical regions worldwide.

Available as poster on the enclosed Public Health CD-ROM.
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Dear Readers,

Dengue is rapidly becoming the most important mosquito-borne disease in the world; a fact in striking contrast with the disease often being dubbed a neglected tropical disease along with related arboviral diseases (e.g. Chikungunya). Looking at the figures, however, dengue cannot be neglected. Estimates for population at risk and numbers of cases per year vary (from 1 to 2.5 billion people at risk, and 50 to 100 million, perhaps even 400 million cases annually), but it is clear that the disease burden is huge. However compared to malaria, there is no “Roll-Back-Dengue Initiative”, no “Global Fund to Fight AIDS, TB and Dengue”, nor even a large bilateral program to sponsor the fight against this mosquito-borne disease.

Dengue is a modern disease. Modern in the sense that sustainable human-mosquito cycles only developed several hundred years ago. And modern in the sense that its vectors, and hence transmission, thrive with urbanization, population growth, global travel, and our irresponsible disposal of modern products (plastic, used tires). As a result, dengue is spreading, moving north – an alarming trend that has now reached Europe.

The WHO and an international group of experts held a series of consecutive meetings in 2011 and 2012 to draw up a roadmap for the control of dengue and to make it a priority for the WHO. It might be believed that we know most tricks about mosquito control, but the Aedes vectors are tricky due to their biology and behaviour. This is a classic field to apply integrated vector management (IVM) to support integrated disease control (IDC). Besides good practice in vector control, we refer to the scientific work on transgenic mosquitoes and progress in developing vaccines. Using the right diagnostics helps target vector control to the areas where the problem is. For Bayer, dengue vector control is as important as malaria vector control and, together with partners, we are committed to developing a strategy for a successful approach.

In past issues of the Public Health Journal, we have highlighted the benefits of long-lasting insecticide-treated bednets, which remain valid. Nevertheless a word of caution needs to be raised about the increased risk of flammability and the resulting risk of being injured by a net catching fire. We took that risk seriously and performed tests with the three netting materials. We can report now that LifeNet® (polypropylene) is classified as “non-flammable”, whereas PE and PET are listed as “easily flammable” according to the Norme Française.

Although not old enough to have benefited from the famous CARE packages which CARE USA distributed after the second world war in Germany, I know the expression by heart from parents’ stories. Even today, “care package” is used as a synonym for helping someone in a difficult situation. In this issue of the Public Health Journal, we acknowledge CARE’s leading role as in emergency relief and health projects with a portrait of Care Germany / Luxemburg.

I wish you pleasant reading.

Gunnar Riemann

Member of the Bayer CropScience Executive Committee and President of the Environmental Science Division Worldwide
Dengue is a viral disease transmitted by mosquitoes (Aedes sp.). The virus is present in most parts of the world with four different serotypes. The vector mosquitoes feed almost entirely on humans, mostly during daylight hours, both outdoors and indoors and do not fly beyond 100 meters from where they emerge. The mosquitoes benefit from city life, travelling, and global warming: With an exploding number of new urban areas, and over 2.5 billion air travelers per year, plus global trade offering round the world free transport for the vector’s eggs, dengue is thriving.

2.5 BILLION PEOPLE live in dengue endemic areas and the list of countries suffering outbreaks in the last months alone is overwhelming: Argentina, Brazil, Paraguay, Bolivia, Cayman, Singapore, Philippines, Vietnam, India, Polynesia, Kenya, Salomon, Borneo, Thailand, Portugal, Colombia, Costa Rica, Vanuatu, Florida... and more.

WE ARE NOW just starting to measure the economic impact of the disease. A study across several countries suggests a direct cost ranging from US$ 600 to US$ 1,500 per person/case, depending on ambulatory treatment or hospitalization, and on the country’s standard of living. With more frequent outbreaks, and therefore exposure to the several virus serotypes, the severity and mortality of dengue will increase, calling for more trained personnel in hospitals to care for the patients. With an estimate of 50 to 100 million cases a year, the burden of the disease is staggering. Moreover, some specialists suggest that what we see is probably only the tip of the iceberg.

CURRENTLY, the most advanced vaccine still has significant limitations, only controlling three of the four virus strains; but it is in a late phase of development. The best available preventative measure is to reduce, as much as possible, the risk of being bitten. Classical vector control consists of larviciding and space spray; but unless conducted systematically and regularly, they do not prevent sporadic outbreaks. There is a strong need to try and test new paradigms and to consider an integrated approach, which combined with entomology, community engagement, and epidemiology could lead to better impact of dengue prevention programs.

FOR BAYER, the fight against dengue is a priority and we strongly advocate an integrated approach combining sustainable vector control measures with surveillance systems, and future vaccination implementation. In several parts of the world we are engaging with experts in these fields and together are willing to learn and explore new solutions for the future.

Much remains to be invented, and Bayer is proud to be part of this global challenge!
Nearly half the world’s population is at risk of dengue disease. Today it is the fastest growing global health problem caused by a mosquito-borne disease. Despite this, dengue receives much less attention from most sectors than many other tropical diseases. It is essential that this changes. Integrated vector management of the primary disease carrier *Aedes aegypti* must be improved, as well as ways to combat the Asian tiger mosquito (*Aedes albopictus*), which is spreading rapidly to new habitats and has become an efficient vector of the dengue-related disease chikungunya.
*Aedes* mosquitoes have adapted perfectly to the modern world. Ever since the Second World War, increasing mobilization of people and goods has helped them spread around the world. Rapidly expanding urban areas provide them with optimal habitats close to their preferred host – humans. Accumulating disposable items such as cans, plastic containers, and used tires collect water for them to breed in, and the eggs can be transported anywhere in the world by growing global trade. Climate change means many more regions are becoming suitable for imported mosquitoes to establish local populations.
Aedes mosquitoes are vectors for dengue as well as other arbovirus diseases such as yellow fever and chikungunya. These widely distributed mosquitoes – which include the most efficient disease vectors *Aedes aegypti* and *Aedes albopictus* – have adapted their life cycles to conditions of human domestication, and have spread in both developing and developed countries. Factors that favor their spread are mostly modern developments that expand their range of habitats, such as urbanization, particularly unplanned, and globalization with international movement of goods. Not only mosquitoes can travel anywhere in the world within 48 hours, but rapid mobilization of people means infected humans can introduce the virus to local mosquito populations. Finally, climate change can create warmer weather, which favors mosquitoes invading temperate regions or higher altitudes, and higher rainfall can provide more egg laying sites.

A recent disease

Dengue is a fairly recent human disease, first arising in West Africa when one of the four viruses that currently cause dengue fever crossed species from monkeys to humans some 500 years ago. But until the Second World War the disease was relatively rare. Fatal forms of the disease, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), first arose only 55 years ago during an epidemic in Manila.

Dengue fever is caused by four different, but closely related flavivirus species that are also related to yellow fever and Japanese encephalitis viruses. Since these dengue viruses are different, infection by one induces life-long immunity to that virus, but not to the other three. Infection with one dengue virus tends not to be lethal, but leads to a chronic disease associated with high morbidity, which contributes to continuing poverty in dengue endemic regions. A second infection with one of the other dengue viruses causes more severe symptoms that can lead to DHF or DSS. In highly endemic regions such as South East Asia the risk of infection with multiple virus types before children reach adulthood is extremely high. The appearance of a new virus type in regions where transmission was relatively low can rapidly lead to a new epidemic. This is compounded by the fact that the viruses are continually evolving to become more virulent, i.e. becoming more efficient at infecting mosquito vectors.

Increasing severity

Over the last few decades dengue has not only spread geographically, for instance re-emerging in South America, but it has also increased in severity. Between 1970 and early 2000 only serotypes 1 and 2 were present in the western hemisphere, while all four types were only found in Asia. By 2004, all serotypes were present in almost all dengue endemic regions throughout the world, as well as different variants of dengue 2 arising from Asia. This has resulted in epidemics becoming more frequent and much more severe in Latin America and Asian Pacific regions.
In 2010, all six WHO regions recorded dengue fever, since for the first time indigenous outbreaks were also reported in Europe. Estimates of incidence in Africa are severely underrepresented since febrile diseases are often attributed to malaria or other causes. Today possibly as many as 2.5 billion are at risk from dengue, throughout almost all tropical and sub-tropical regions of the world, and 500,000 severe cases of DHF occur each year.

A debilitating disease

Chikungunya virus (CHIKV) is an alphavirus of the family Togaviridae also transmitted by Aedes mosquitoes. Although not usually fatal, it causes severe, sometimes prolonged suffering. Symptoms are initially similar to dengue, with a high fever lasting several days, joint pain, muscle pain, headache, nausea, fatigue and rashes. Joint pain can last for weeks, months or even years. There is currently no treatment nor approved vaccine.

Chikungunya, meaning “to become contorted” in the Kimakonde language to describe those suffering from joint pain, was first identified during an outbreak in Tanzania in 1952. Today outbreaks occur in Africa, Asia and the Indian subcontinent. Originally transmitted by Ae. aegypti, CHIKV has adopted Ae. albopictus as a secondary vector. These mosquitoes have spread to Europe and the Americas over the last few decades. Local transmission of CHIKV was reported for the first time in Europe in 2007 during a local outbreak in north-east Italy (see page 18).

Role of vaccines

Unlike other vector-borne diseases, preventing dengue or chikungunya depends entirely on vector control. Hopes are of course pinned on developing effective vaccines against these diseases. In the case of chikungunya preliminary vaccine research showed some success, but was not continued due to lack of funding.

DISTRIBUTION OF DENGUE

Endemic throughout tropical and sub-tropical regions worldwide, documented outbreaks of dengue are indicated, including isolated incidences in the USA, Europe, and Russia. Little is known about dengue outbreaks in Africa.
Vaccines for dengue face the challenge that they must neutralize all four virus types. At least nine tetravalent vaccines are currently under development, and a number of candidates are already in clinical trials with humans. The ideal vaccine should provide single-dose life-long protection against all four viruses, which will take many years to determine. Even then, as history has shown with yellow fever or Japanese encephalitis, immunization control has limited success unless transmission rates are also reduced. Despite having a yellow fever vaccine since 1937, epidemics still occur, for example in Uganda in 2011, although these are not as severe as 100 years ago.

Realistically, a vaccine will not eliminate dengue. Rather the role of vaccines should be seen as an additional tool that needs to be properly integrated and applied to disease control. Vaccine availability will likely be limited, for example Sanofi’s first five years supply will not be enough to cover Brazil’s needs alone. Vaccination programs will also be expensive and have the greatest chance of success when programs are sustained over the long-term in populations that will benefit the most. It will be essential to lower transmission to levels where vaccine control can be more effective. This means that integrated vector management (IVM) strategies will be essential to ensure the success of a vaccine combined with integrated disease management (IDM) – and vaccines suppressing transmission should help make vector control more effective.

**Vector control**

Vector control is complicated by the *Aedes* mosquito’s synanthropic life style and diurnal activity, making it difficult to find any one intervention point or personal protection measure that is highly effective, such as insecticidal bednets for malaria. Existing vector control programs are based on larval control, limiting biting adults, and vector population control, ideally integrated into IVM.

*Aedes aegypti*, the primary vector of dengue, has evolved its life cycle to fit the niche of human habitation, taking advantage of domestic water supply and waste containers for laying eggs. The female mosquito feeds during the day, particularly in the morning and evening, often biting many different people at a time, both outdoors and indoors.

*Aedes albopictus* (Asian tiger mosquito) is primarily a vector of dengue in China and Asia, and increasingly an efficient vector of chikungunya. It has spread to North America and parts of Europe due to its tolerance to low temperatures, even below freezing, and its ability to hibernate or shelter in micro-habitats. The female mosquito bites in outdoor locations during the day.

**Source reduction and larvicides**

The most widely used method of vector control is source reduction to prevent mosquitoes laying eggs. Physical removal of breeding sites works well, but relies on community commitment, altering human behavior through education, and above all improving clean water and sanitation infrastructures along with urban planning. Breeding areas that cannot be eliminated can be treated with insecticides that target mosquito eggs, called larvicides. Recent new methods not relying on insecticides are being tested, including releasing sterile insects or *Wolbachia*-infected insects refractory to dengue virus (see page 35).

The use of larvicides is usually restricted to water containers that cannot be eliminated or managed.
through environmental control programs. Larvicides used in water-storage containers should not change the taste, odor or color of the water, nor be toxic to other species. However adding insecticides to domestic water may be unacceptable, particularly if this is also a source of drinking water. Sometimes sites are difficult to find, particularly the common breeding sites of *Ae. albopictus*, which prefer more natural habitats such as holes in trees and forest puddles. Identifying the most productive breeding sites requires close monitoring of environmental and entomological factors affecting local mosquito populations.

**Adulticides**

Methods of control that target adult mosquitoes use insecticides to reduce vector population size and disease transmission, usually in response to an epidemic. Such adulticides are applied as either residual surface treatments or space spraying, according to recommendations for low-risk use and sound management by the WHO Pesticide Evaluation Scheme (WHOPES).

**Residual treatment**

Suitable insecticides with both adulticiding and larviciding effects can be applied with hand-operated compression sprayers. Indoor residual spraying (IRS) involves applying insecticides to the walls and roofs of all houses and domestic animal shelters in a specific area. The insecticides kill the adult vector mosquitoes that land and rest on these surfaces.

**Space sprays**

Recommended only in emergencies, space spraying is used to suppress an ongoing epidemic or to help prevent one that seems likely. Space spraying is intended to rapidly reduce the number of infective adult mosquitoes for a short time to reduce virus transmission. It is not clear whether the short-term effect of space sprays has any impact on insect populations over a longer term. In the early stages of an epidemic it may provide some relief and time for implementing larviciding and community-based source reduction to provide more effective long-term vector control.

**Insecticide-treated materials**

Due to the daytime activity of *Aedes* mosquitoes, the use of insecticide-treated nets (ITNs) or long-lasting insecticidal bednets (LNs) in dengue control has not been well studied. This is compounded by the fact that while ITNs may be distributed in rural areas where malaria is endemic, they are often not used in areas at risk from dengue. In Vietnam, for example, most rural children sleep under ITNs, but in urban areas where malaria is not endemic, they mostly sleep under untreated nets or no nets at all. Untreated nets provide no

**DURING AN EPIDEMIC**, space spraying can rapidly reduce adult mosquito numbers, providing some relief before implementing effective long-term integrated vector control.
protection against dengue, and children taking a midday nap under untreated nets are just as likely to be hospitalized for DHF as those sleeping under no nets\(^1\). However, where ITNs have been tested, they did provide good personal protection against dengue and chikungunya for those who sleep during the day, such as sick or older people, nightshift workers, and infants.

ITNs were also demonstrated to reduce *Ae. aegypti* mosquito populations and dengue transmission in a trial carried out in Haiti\(^2\). Using LNs at high-level coverage means they were acting as vector control tools. Since then the use of insecticide-treated materials (ITMs) has been extended to textiles such as curtains hung in windows, doorways, corridors, cupboards, and wardrobes, netting used as room dividers or eave linings, or insecticide-treated covers for water containers, jars and large domestic water storage barrels (see PHJ No. 22). Recent trials confirm that ITMs can have a dramatic impact on vector populations, and are well accepted by target communities. However, their implementation depends on a number of favorable conditions such as house design and it has yet to be established whether they sustainably reduce dengue transmission.

**Insecticide resistance**

The development of resistance to insecticides poses a serious threat to sustainable vector control, since only a few insecticide classes with different modes of action are available. Pyrethroids and organophosphates are the major classes used in direct dengue control, both are used in space spraying. Residual spraying with carbamates plays a minor role in adult *Aedes* mosquito control, while organophosphates are also used as larvicides. However, in terms of volume, pyrethroids are used the most, since they are also the only class of insecticide approved for ITMs.

Resistance to pyrethroids is reported to be widespread in *Ae. aegypti*, but less prevalent in *Ae. albopictus*. However, data on resistance are mostly derived from a variety of techniques and bioas-
says, making comparisons very difficult. Moreover, laboratory susceptibility tests say nothing about the potential impact of resistance on actual vector control programs, nor substitute for evidence-based decisions about insecticide use and resistance management.

Obviously *Aedes* mosquito control urgently needs better coordinated data, monitoring, tests, and tools to implement effective integrated resistance management (IRM) strategies. Such strategies must aim to maintain the efficacy of all insecticides classes by using mixtures or rotations. At the moment, the first indications to change to another insecticide are usually when a control program fails. This means local resistance has already reached high levels in the population and it may take many years before this insecticide class can be used again. With so few insecticide classes available, this is an unacceptable waste of resources. To ensure all insecticides remain effective in dengue control programs, IRM must be a vital component of IVM.

**Ways forward**

Perceived ineffectiveness of vector control is mostly due to the fact that strategies in different countries, and even within countries, are usually quite diverse, inefficient, uncoordinated, under-funded, unsustained, lack community participation or political commitments, and rely on different authorities for organization and funding (health, environment, community). Sustainability and targeting of vector control is generally a problem in urban settings, but particularly when there is no strategic plan or guidelines.

This highlights the urgent need for innovative strategies and policy directions for effective vector control interventions, as urged by the WHO. Together with the private sector, Bill & Melinda Gates Foundation, and Centers for Disease Control, the WHO has now also engaged partners in the Global Collaboration for Development of Pesticides for Public Health to focus specifically on the problem of dengue and dengue vector control\(^3\) (see page 14).

Avril Arthur-Goettig

**CONCLUSION**

Combating virus diseases transmitted by *Aedes* mosquitoes urgently needs IVM and IRM programs that incorporate an innovative portfolio of larvicides, adulticides and insecticide-treated materials. Just as communities such as the Roll Back Malaria (RBM) partnership was founded in 1998, now is the time to create a comparable global network of stakeholders to implement coordinated action against dengue and chikungunya diseases.

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**Sources**


WHO global strategy for dengue prevention and control

How to reverse alarming trends

Dengue is one of the 17 neglected tropical diseases designated by the WHO as priorities in a roadmap to overcoming the suffering caused by these ancient diseases. But dengue can no longer be considered tropical or ancient, rather the most rapidly spreading mosquito-borne disease today; nor can the world afford to neglect it. This is why the WHO has made dengue a priority, and published a detailed strategy for combating dengue over the next eight years.

For some time the WHO has been scaling up activities to reduce the global burden of neglected tropical diseases (NTDs). In 2005, the WHO established the “Department for Control of Neglected Tropical Diseases” (WHO/NTD). Subsequently, in 2011, the WHO drew up a roadmap on: “Accelerating Work to Overcome the Global Impact of Neglected Tropical Diseases”.

It was already apparent that dengue had shifted into a category of its own, as an emerging vector-borne global disease. The threat of dengue was recognized in 2011 when a WHO regional committee passed a resolution on “Dengue: call for urgent interventions for a rapidly expanding emerging disease”. In 2010 all six WHO regions recorded dengue incidences, including the European Region.

GCDPP Meeting

At the 8th GCDPP Meeting at WHO headquarters in Geneva, 20-21 February, 2012, the message was that dengue control is now one of the WHO’s major priorities. Hiroki Nakatani, WHO Assistant Director-General, HIV/AIDS, Tuberculosis, Malaria and Neglected Tropical Diseases, stressed that efforts to combat dengue address several Millennium Development Goals, such as combating HIV/AIDS, malaria and other diseases, eradicating extreme poverty and hunger, and providing safe drinking water. Poverty, unplanned urbanization, and inadequate clean water or waste disposal have always been closely associated with dengue disease.

The objectives of the GCDPP meeting were introduced by Morteza Zaim, Coordinator, WHO Vector Ecology and Management:
• To review the evidence on effective dengue vector control interventions (both during outbreaks and as sustained control interventions in endemic countries).
• To gather more evidence on innovative technologies in the pipeline.
• To review the integrated vector management (IVM) approach to dengue management, including integrated vector and case surveillance.
• To recognize the role of vector control in an integrated program before and after the introduction of a dengue vaccine.
• To identify and acknowledge the role of partners in dengue control.

Urbanization and poverty

Chairing the meeting, Ronald Rosenberg, Associate Director of Science, U.S. Centers for Disease Control and Prevention, reminded everyone that about 1 billion people are at risk of dengue virus infection, with over 50 million new infections per year*. Moreover, 75 years of persistent transmission in Florida means even developed countries are at risk. The main vectors of dengue viruses, *Ae. aegypti* and *Ae. albopictus* have evolved to breed close to human habitation. Now, for the first time in history more people live in urban areas than the rural population, bringing greater numbers of people into contact with the vector and virus.

The mosquito *Ae. albopictus*, a vector of both dengue and chikungunya, is the predominant vector for dengue in Asian countries and China. Known as the Asian tiger mosquito, it has now spread to the Americas, Africa, and Europe. In part, this is due to international trade in used tires, one of the mosquito’s favorite breeding places. Changes in weather patterns have also extended the north-south geographical distribution of this vector, which is able to withstand lower temperatures than *Ae. aegypti*. The Asian tiger mosquito is responsible for recent indigenous outbreaks of dengue in France and Croatia in 2010 (see page 18).

Globalization and climate change

This highlights how international travel and trade, migration of people and mosquitoes, and warmer weather are now new factors driving the resurgence of dengue disease. To make matters worse, as Rosenberg explained, unlike other vector-borne diseases, increasing spread of dengue infections is linked with enhanced disease severity due to coinfection with different virus serotypes. Immunity to one virus serotype does not protect against the other three virus types. Co-, or re-infection increases the incidence of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), the most serious forms of the disease.

* Other estimates reach up to 2.5 billion people at risk and 50 to 100 million cases a year (see page 5 and 9), and according to Simon Hay (Oxford University) even 400 million cases might be realistic.

**USED TIRES** provide perfect breeding locations for dengue vectors.
Indeed, dengue hemorrhagic fever, almost unknown 50 years ago, is now common in South America and the Caribbean. In contrast, in Somalia, dengue incidence exceeded 50% among peacekeepers who reported acute fever, meaning that dengue in Africa is probably severely under-reported, usually obscured by other diseases such as malaria.

Vital role of vector control

So far, vector control is the only method of controlling transmission of dengue. Although at least nine candidate vaccines are in development, the challenge is that a successful vaccine must protect against all four virus serotypes. It will take a number of years before it is known whether a vaccine is really effective, warned Rosenberg. Even then, vector control will continue to play a vital role in reducing transmission rates to allow successful vaccine coverage. Lessons have been learned from polio and measles campaigns, or the yellow fever vaccine, available since 1937, which has reduced epidemics, but not eliminated the disease.

In reverse, when a successful vaccine becomes available in the near future, this will provide added impetus to vector control. As transmission rates start to fall, vector control will become more effective, as well as essential for preventing new outbreaks of the disease.

Integrated vector management (IVM)

Countries should adopt the integrated vector management approach to vector control as promoted by WHO. Defined as a rational decision-making process to optimize the use of resources for vector control, it aims to improve efficacy, cost effectiveness, ecological soundness and sustainability of vector control interventions. Dengue vector control is most amenable to the implementation of the principles of integrated vector management, which ensure judicious use of insecticides in combination with other interventions.

Effective vector control measures are critical to achieving and sustaining reduction of morbidity attributed to dengue. Preventative and vector control interventions aim to reduce dengue transmission, thereby decreasing the incidence of the infection and preventing outbreaks of the disease.

Increasing population, mobility and morbidity

Invited officials from national ministries or institutes of health presented perspectives from their countries. In Brazil, a shift in virus serotypes has led to escalating numbers of severe cases of DHF and DSS, particularly in children, and more dengue-associated deaths. In China, the first confirmed outbreak was in 1978. The recent geographical spread is probably due to socio-economic factors leading to increased urbanization, climate change, and more international contact. In Indonesia the first report of dengue was in 1968; in 2011 there were over 50,000 cases. In Saudi Arabia the first reported case in 1993 was followed by an epidemic in 1994. Numbers of cases dropped for a decade, but since 2007 incidences have continue to increase.

Keeping up to date

Singapore has performed house-to-house checks since the 1960s, charging fines for not adhering to proper mosquito breeding site prevention. This succeeded in significantly reducing dengue incidences in the 1970s. When in the 1980s transmission started rising again, and after an outbreak in 2005, it was apparent that vector control programs were out of date. Since then constant updating of surveillance and vector control technologies have reduced annual dengue cases and provided early warning, and hence mitigation of outbreaks. This provides a working example of how constant updating is needed to cope with environmental and demographic changes. In Singapore the challenges are clear: the population increased from 2.1 million in 1970 to 5 million in 2009, and
The worldwide incidence of dengue has increased 30-fold over the last 50 years, and more countries are now reporting fresh outbreaks as it spreads to previously uninfected areas. The world must now move from reactive emergency responses to proactive long-term prevention strategies. Dengue must be controlled through significantly improving surveillance, case management, and sustainable vector control strategies, as outlined by the WHO.

**CONCLUSION**

The worldwide incidence of dengue has increased 30-fold over the last 50 years, and more countries are now reporting fresh outbreaks as it spreads to previously uninfected areas. The world must now move from reactive emergency responses to proactive long-term prevention strategies. Dengue must be controlled through significantly improving surveillance, case management, and sustainable vector control strategies, as outlined by the WHO.
Relevance of dengue and chikungunya in Europe

Re-emerging diseases

An epidemic of dengue was declared on the island of Madeira at the beginning of October 2012. The first sustained transmission of dengue in Europe since the 1920s, the following months saw over 2,100 cases among island residents and travelers returning home. Together with recent epidemics of another mosquito-borne disease, chikungunya fever, such outbreaks highlight the increasing threat of vector-borne diseases in Europe.

Sporadic cases of dengue in Europe are not uncommon when travelers returning or visiting from dengue endemic countries develop symptoms after arrival. The rise in dengue transmission globally over the last 2-3 decades and growing worldwide mobility of people and goods have played an important role in increasing these numbers in recent years. Usually it is thought unlikely that European travelers can transfer dengue in their home country since the mosquito vector is not present.

*Aedes aegypti*, the main vector of dengue, is generally not found in EU countries. However, these mosquitoes have been reported in the Netherlands; in 2010 adult mosquitoes were discovered at some used tire companies, and these were related to mosquitoes breeding at a tire company in Florida, USA. *Ae. aegypti* is also found in southern Russia and neighboring Georgia.

Dengue cases in France

In 2010, a few locally transmitted, or autochthonous cases of dengue were reported in France and around Dubrovnik in Croatia; these were transmitted by *Aedes albopictus*, the Asian tiger mosquito. *Ae. albopictus* has been on the increase in EU countries since 1970, also through international trade in used tires. This mosquito has become established in Albania, Bosnia-Herzegovina, Bulgaria, Croatia, France, Greece, Italy, Monaco, Montenegro, the Netherlands, San Marino, Serbia, Slovenia, Spain, Switzerland and the Vatican City. *Ae. albopictus* was also introduced, but did not become

**DENGUE IN MADEIRA** spread rapidly through local *Ae. aegypti* mosquitoes, infecting over 2000 people between October and December 2012.
established in Belgium in 2000 and Germany in 2007 and 2011. The tiger mosquito is not only associated with dengue transmission, it is now a vector for the disease caused by Chikungunya virus (CHIKV). However, CHIKV is still a fairly recent emerging endemic disease and is often misdiagnosed as dengue fever.

Dengue outbreak in Madeira

On October 3, 2012, the Autonomous Region of Madeira (RAM) reported an outbreak of two cases of dengue. Portuguese authorities immediately started implementing measures to prevent further spread and limit the outbreak. Sea ports, airports and all planes leaving the island were subjected to disinfestation. Detailed recommendations were issued on safety measures for blood, tissue, or organ donations from Madeira, mainland Portugal, and recent visitors. The many visitors to Madeira mean that sporadic cases are not unexpected. What was unexpected was the rapid spread and size of the outbreak, due to local *Aedes* mosquitoes. The mosquito population has expanded rapidly and has been difficult to eliminate due to the mountainous topography of the island and numerous breeding sites.

On October 10, due to the unprecedented public health event posed by the autochthonous dengue outbreak in Madeira, the European Center for Disease Prevention and Control (ECDC) published a rapid risk assessment in consultation with the

**Aedes albopictus** show to be increasingly present in various European countries (June 2012, marked in red), whereas the distribution of *Aedes aegypti* is sporadic and localized in Europe. (Source: ECDC report on dengue cases in Madeira 2012).
WHO\(^1\). The most efficient transmission vector for dengue fever, the mosquito *Ae. aegypti*, apparently established a habitat on Madeira in 2004–2005. Portugal’s Secretary of Health said that mosquitoes carrying dengue virus probably came from a tropical country earlier in the year. However, it is unclear whether mosquitoes arrived by air or sea, as Madeira is a popular port for cruise ships. Analysis of the viral genome from several dengue patients in Madeira showed high similarity to DEN-1 serotype viruses currently found in Venezuela and Colombia.

### Reducing risks

The ECDC advised residents to lessen the risk by reducing larval breeding sites in or near households, and recommended that residents as well as visitors take measures to prevent mosquito bites. Since *Ae. aegypti* mosquitoes are active during the day, precautions and protective measures, such as using repellents, should be used throughout the day.

Anyone with suspected symptoms such as high fever and ocular, muscular and joint pain should report to a medical center. However, the ECDC added that 40 to 80 percent of cases are mild or asymptomatic, which poses a threat to blood donations and fully curbing transmission.

Nearby areas such as the Canary Islands and other EU countries were warned to introduce surveillance activities to assess the risk of *Aedes* mosquitoes establishing themselves in other areas.

By October 22, 2012, the ECDC had sent a team of experts to the island to help assist the health authorities in their efforts to control the outbreak. On October 29, the ECDC introduced a new surveillance system integrating hospital and healthcare center data with symptom onset dates and geographical locations. This marked the start of a decline in new cases. By mid-December 2012 the total number of cases had reached 2,050 among residents of the island, and 121 people had been treated in hospital. However, there were no deaths, unlike the dengue epidemic in Greece in 1927–1928, which had a high mortality rate.

### Cases in other EU countries

An additional number of dengue cases spread to other countries in Europe through travelers returning from Madeira. Most cases were in the UK and Germany, with several in France, Finland, Croatia, Denmark, Norway, Slovenia, Spain, Sweden and Switzerland as well as mainland Portugal.

The ECDC published an updated rapid risk assessment on November 20, outlining the measures implemented so far\(^2\). However, they warned: If dengue-transmitting mosquitoes survive the winter, this will increase the risk of spread to continental Europe in summer 2013.

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Chikungunya fever

For the first time, 2004, Chikungunya virus (CHIKV) infections re-emerged with an efficient mosquito-human-mosquito cycle in Lamu (Kenya) with 13,500 cases, then a second outbreak in Mombasa. Subsequently CHIKV spread eastward through islands in the Indian Ocean. Over 225,000 infections occurred in the Union of the Comoros, with CHIKV detected in *Ae. aegypti* mosquitoes.

*Ae. albopictus* as new virus vector

Moving on to La Réunion, at the peak of the epidemic 40,000 CHIKV cases were being reported each week, resulting in an estimated total of 266,000 cases. Now, the disease was associated with a number of neurological symptoms, prenatal infections and death.

The major shift was that the virus was now being transmitted by *Ae. albopictus*. A mutation in the virus had increased its infectivity in *Ae. albopictus*, which accounted for the size and distribution of the outbreaks.

Otherwise autochthonous transmission would not have been possible on La Reunion, where *Ae. aegypti* are not found. The significance of the vector shift means that CHIKV could now spread to areas where *Ae. albopictus* is found, such as Europe.

First, the virus spread to India where it resulted in millions of cases, and infection still continues. By 2007, the virus had been imported into at least 18 countries in Asia, North America and Europe, and even developed into local autochthonous transmission of CHIKV in Italy.

**Chikungunya outbreak in Italy**

Following reports of unidentified cases of fever and joint pain in two nearby villages in northeastern Italy, tests were carried out to identify the source of infection and modes of transmission. Locally captured mosquitoes and blood samples from patients were analyzed by PCR and serological assays to identify the causal agent, which proved to be CHIKV. Between July 4 and September 27, 2007, a total of 205 cases of CHIKV disease were recorded, fairly mild in almost all cases, and only one reported mortality. The outbreak was thought to have started when a man from India developed symptoms while visiting relatives in one of the villages. Local *Ae. albopictus* mosquitoes were responsible for the autochthonous transmission.

This outbreak highlighted for the first time the potential of the virus to move to novel ecological niches, including Europe. The risk of CHIKV spreading into new areas is a constant threat, due to the high levels of virus in infected humans, which promotes human-mosquito-human cycles in areas where the mosquito vector has established a habitat.

**CONCLUSION**

The outbreaks of locally transmitted dengue or CHIKV diseases in Europe emphasize a new threat of re-emerging infectious diseases spreading in this era of globalization and climate change. Recurring or ongoing epidemics in many parts of the world, and the increasing geographical distribution of the mosquito vectors that can transmit these diseases increase the chances of more autochthonous outbreaks in the future.

**Article on the enclosed Public Health CD-ROM**
From 1995 to 2004 only 699 dengue cases and five deaths were reported from four districts of Pakistan. Between 2005 and 2011 numbers of confirmed cases dramatically increased to 55,946 and deaths to 539, affecting 105 out of 146 districts. Since then the disease has become widely accepted as one of the major public health problems in Pakistan.

*Aedes aegypti* and *Ae. albopictus* are considered the major vectors of dengue and both species, particularly *Ae. aegypti*, are found to be associated with man-made clean water domestic habitats in human dwellings. Similarly, both species have also exhibited a well-defined rising trend in population in post monsoon seasons (September to November). During 2005 to 2011, a 95.5% caseload in the country was reported during these months, and cases rapidly declined after November, which indicated a possible positive correlation between vector densities and disease incidence.

The year 2011 had one of the most severe outbreaks of DF, affecting people of all ages and sexes in Pakistan, particularly in Punjab. This outbreak was ranked as the worst, since it affected almost 0.6 million people in a span of 72 days, resulting in 22,198 confirmed cases and 378 deaths, mostly in Lahore-Punjab.

**Why vector control is important**

Vector control has proven highly effective in preventing disease transmission around the globe. However, implementation of a single intervention has not yielded the desired results, and ideally one or two even more interventions should be used simultaneously, also through involvement of different departments. This approach is called integrated vector management (IVM), which is based on the principle that effective and sustainable vector control is not the sole responsibility of the health sector, but requires collaboration between various public and private agencies, line departments and community participation.

From 2009 to 2010 the prevention and control program in Pakistan has been based on the use of carpet fogging and indoor residual spraying (IRS) with pyrethroid insecticides as the frontline defense to combat the problem in country. However...
Since 2005, in Pakistan, dengue is the fastest emerging arboviral infection. Lack of inter-sectoral coordination, planning, trained human resources, surveillance systems and effective monitoring and evaluation are the major current challenges to designing evidence-based, cost-effective, community-friendly and sustainable dengue vector control strategies. In 2011/12, IVM strategies were optimally implemented in Pakistan, particularly in the Punjab province, which proved outstandingly successful in controlling dengue fever and dengue hemorrhagic fever (DF/DHF).

Implementation of IVM strategies

In the light of lessons learnt from regular outbreaks of DF/DHF in the country, particularly in Punjab in 2011, the Directorate of Malaria Control, Ministry of Inter-Provincial Coordination, and Government of Pakistan developed evidence-based country-specific national guidelines for dengue vector control through a consensus of all stakeholders and gathering best knowledge and practices available in the world. The central theme of these guidelines was promotion and implementation of an IVM strategy in Pakistan, which has been endorsed as the recommended strategy to exploit the preventive power of vector control in cost-effective, sustained and ecologically sensitive ways.

These guidelines provide practical help to local authorities for strengthening their capacities to interrupt disease transmission by reducing vector densities through a functional mechanisms of inter-sectoral coordination for cost-effective and
sustainable dengue vector control initiatives in local settings. Provincial governments, particularly the Government of Punjab implemented an IVM strategy according to best principles, and set an example of its effectiveness in control of DF/DHF through the following key steps:

**Legislation:** Under the direction of the Chief Minister, the Punjab Health Department drew up a comprehensive legislative policy (Law) to take immediate regulatory measures to prevent and control the epidemic of DF, under the West Pakistan Epidemic Disease Act entitled “The Punjab Prevention and Control of Dengue Regulations, 2011”. This legislation covers all areas of solid waste management, environmental management, biological control, quality assurance of insecticides, intersectoral coordination, strengthening disease surveillance, capacity building, community participation, empowering local authorities and imposing a penalty in the case of failing to follow the legislation.

**Coordination:** Very effective and productive inter-sectoral coordination mechanisms involving all stakeholders other than health (district governments, municipalities, departments of education, irrigation, livestock, water and sanitation authorities, etc.) were developed. Weekly meetings were held for long-term planning and sharing responsibilities. Dengue was included in the curriculum at school and college levels. Rescue 911, scouts, cooperatives and housing societies were taken on board. Free help lines were started to advise, recommend, and answer dengue-related issues. The Department of the Environment regularly inspected tire shops to provide guidance for proper management of used tires.

**Partnership building:** A very strong and effective partnership was established with the private sector, involving civil society, NGOs and donors for resource mobilization and effective implementation of IVM strategies. In this regard, seminars, workshops, and symposia were organized through this Public-Private-Partnership. Partnerships were established with other countries (Sri Lanka, Malaysia, Singapore, etc.) that have extensive experience in dengue control. Experienced scientists from these countries were invited to deal with the situation and build up the capacity of local staff.

**Human resource development:** Highly qualified technical personnel including entomologists, epidemiologists, environmental inspectors, community mobilizers, etc., were recruited for regular disease surveillance throughout the years.
**Capacity building:** To build up and strengthen the capacity of newly recruited and existing personnel, a comprehensive plan of action was prepared. At the end of the epidemic 98 people were sent abroad (Thailand) to be trained as Master Trainers. For sustainability of the initiative and also to pass down the impacts of these capacity building programs, these Master Trainers conducted trainings at the grass root level as a part of overall District Implementation Plans (DIPs) for dengue control.

**Establishment of technical working groups:** Technical working groups (TWGs) were established for situation analysis and development of evidence-based recommendations.

**Highest level political commitment:** The Chief Minister of Punjab, along with his whole cabinet, actively participated in anti-vector campaigns, holding meetings on a daily basis with all stakeholders to ensure the implementation of legislations. This level of political commitment also enhances community participation and mobilization of human and financial resources.

**Long-term planning:** To ensure the sustainability of all initiatives, a 5-year master plan was prepared, promoting the implementation of IVM strategies. In this regard, Rs 780 million (approx. US$ 8 million) of funds were allocated by the Government of Punjab. Research and development (R&D) was made a fundamental part of long-term planning.

**Establishment of an anti-dengue brigade:** To monitor the implementation of guidelines and newly passed legislation, an “Anti-Dengue Brigade” was established. Thousands of students from schools, colleges, and universities were made volunteer members and played a very active role in community mobilization and awareness raising campaigns. A task force was also developed to monitor and manage breeding sites in graveyards, public fountains, railway junkyards, etc.

**Community motivation and participation:** All political leadership of the provincial government also actively participated in anti-dengue campaigns, holding meetings on a daily basis with their local communities for motivation and participation. Female health workers were also involved in community mobilization.

**Celebration of dengue day and other events:** Special events including dengue day, sports festivals, etc., were regularly organized as a part of overall community awareness campaigns.

**Combination of interventions:** Under the umbrella of IVM, all possible interventions (LLINs for patients in health facilities, IRS around confirmed dengue cases, larviciding and release of fish in all identified breeding places, and above all in/outdoor fogging) were launched through active and effective participation of local communities.

**CONCLUSION**

In Pakistan, like many other countries, dengue has become a major public health issue that requires essential changes in national policy and strategies for ecologically sound, sustainable dengue control. Implementation of integrated vector management (IVM) in the Punjab province during 2012 reduced confirmed cases to only 325 among 789 suspected cases, and no deaths occurred. Outstanding success for dengue control during 2012 in the Punjab provinces demonstrated that IVM is the only and best way forward to control dengue in a sustainable way.
Dengue and chikungunya in Mauritius

Preventing reintroduction of disease

Recent epidemics of dengue and chikungunya in Mauritius activated prevention and preparedness programs based on experiences with eliminating malaria from the island. Surrounded by endemic regions, strategies include planning responses to both epidemic and interepidemic phases. In particular, current measures must focus on preventing reintroduction of these viruses into island vector populations of *Aedes albopictus*. 

Photo: © tranac - Fotolia.com
The Republic of Mauritius comprises the island of Mauritius and three other islands, Agalega Island, Cargados Carajos Shoals and Rodrigues, located in the Indian Ocean off the coast of Madagascar. The island of Mauritius has sloping plains rising from its 117 km of coastline up to central mountains. The population of over 1.3 million depend on an economy based on sugar, textiles, financial services, and especially tourism. Together with other islands such as Madagascar, Réunion, Comoros, and the Seychelles they form the Indian Ocean Commission (IOC), which is striving for greater cooperation with the African Union.

**Local mosquito vectors**

In Mauritius, winter between June and November is warm and dry, while summer is hot and wet, ideal for the vector transmitting the viruses causing dengue and chikungunya: the local mosquito *Aedes albopictus*. Thus the island has a high transmission potential, and has suffered epidemics of chikungunya in 2005 and 2006, and dengue fever in 2009. Once introduced, these diseases are difficult to eradicate. But more importantly, Mauritius has extensive travel and trade links with surrounding endemic regions. Many environ-

**MAURITIUS has an ideal tropical climate for tourists – and also for *Aedes* mosquitoes, the vectors of viruses causing dengue and chikungunya.**
mental pockets on the island harbor local mosquito populations that can pick up and spread imported dengue and chikungunya viruses.

Environmental factors triggering a new epidemic phase are poorly understood, but thought to be related to temperature, rainfall (cyclones), vector populations and the number of people in the population already immune (called herd immunity). For example, a chikungunya endemic tends to take off if more than 30% of the population has never been infected, and are thus susceptible to the disease.

History of anti-malaria programs

To eradicate malaria, Mauritius first initiated extensive measures in the 1940s, subsequently resumed in the 1980s. Thus over many years, local and regional regulators have built up a solid infrastructure for eliminating mosquito-borne malaria. Such efforts reduced annual malaria deaths from 3,000 per year to only sporadic local cases of disease between 1952-1967.

Mauritius then entered a maintenance phase to prevent reintroduction of malaria, screening travelers and migrant workers from endemic areas and providing presumptive treatment for fever cases before diagnostic confirmation. After a large cyclone in 1979, local cases surged again, causing malaria epidemics during the 1980s. The last indigenous case was reported in 1997, and since then an average of 48 imported cases occur each year.

A case study on “Eliminating Malaria: Preventing reintroduction in Mauritius” by the WHO in 2012 documented the long history of malaria control on the island. The main message is that efforts must be maintained after epidemics have been controlled, shifting expenditure from prevention to surveillance. Mobilizing considerable financial resources in Mauritius was ensured by strong political will, leading to long-term, stable financing: Mauritius invested over US$ 45 per capita per year during epidemics and subsequently US$ 2 on maintaining malaria prevention.

Operational plan for chikungunya and dengue

With up to 10,000 cases of chikungunya in 2006, and a dengue epidemic in 2009, Mauritius was faced with challenges similar to malaria control. It immediately imposed the strategies of containment and mitigation during epidemic phases and early warning, surveillance, and focal control during interepidemic phases. To clarify targeted interventions the Ministry of Health and Quality of Life in the Republic of Mauritius detailed an “Operational plan for the prevention and control of chikungunya and dengue in the Republic of Mauritius” in 2009.

The priority for eliminating these diseases involved putting together a preparedness plan that includes outlining procedures for managing outbreaks and coordinating logistics. The overall planning defines the roles of Regional and Local Ministries for the Environment, Local Government, Agro Industry, Food Production and Security, Tourism, Education, Culture and Human Resources, and Social Security, as well as other target groups such as hospital administrators, health directors, public health superintendents and inspectors, surveillance officers, and other stakeholders.

Integrated vector management

The main tool to combat dengue and chikungunya is integrated vector management, combining space sprays (thermal fogging or ULV aerosols), indoor residual spraying (IRS) and larvicides. Routine island-wide entomological surveillance and geograph-
The almost military-like offensive in Mauritius was an important factor for successes in eliminating malaria, dengue and chikungunya. Integrated vector management continues to form the core intervention tool. Data collection, analysis and feedback at every level of the government and health system lead to robust program implementation. Continuation of strict policies is essential for preventing reintroduction, and demonstrates that Mauritius is determined to impose a very low risk of resurgence.

**Preventing reintroduction**

Activities to minimize the risk of importation are at the core of the country’s strategy to sustain elimination and prevent reintroduction. This means protecting ports of entry and screening incoming travelers, particularly those coming from endemic countries. The international sea-port and airport are the only areas treated with space spraying, IRS and larviciding all year round, and all incoming planes are treated with insecticides. Deposits of used tires are also routinely sprayed.

Passenger screening targets and monitors prepared lists of high-risk visitors – surveillance officers proactively screen migrant groups from endemic countries, and revisit them after a few days. Suspected cases are requested to visit nearby health centers; positive cases are rapidly isolated and treated. All contact persons are screened and neighborhoods are immediately treated with insecticides to prevent local transmission. Space spraying is performed over a radius of 300 meters around the case house, and larval surveys for mosquito breeding sites must be carried out over a 500 meter radius within 24 hours of the reported case.

**CONCLUSION**

The almost military-like offensive in Mauritius was an important factor for successes in eliminating malaria, dengue and chikungunya. Integrated vector management continues to form the core intervention tool. Data collection, analysis and feedback at every level of the government and health system lead to robust program implementation. Continuation of strict policies is essential for preventing reintroduction, and demonstrates that Mauritius is determined to impose a very low risk of resurgence.

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Diagnostic methods to detect dengue and chikungunya virus

Accurate and reliable

Dengue or chikungunya can only be reliably diagnosed by various laboratory methods that detect antibodies or isolated virus, and more recently molecular tests to quantify viral genetic material. The most important diagnostic factors are to be as specific, sensitive and fast as possible. Fast-track Diagnostics has developed easy-to-use, real-time PCR test kits that are accurate, reliable, and can identify each dengue virus type, chikungunya, and other fever-causing infections.

The enveloped dengue virus is a positive-sense, single-strand RNA virus of the genus Flavivirus. Dengue viruses consist of four serotypes (Dengue virus types 1-4). The diameter of this spherical virus is about 50 nm with a 30 nm capsid that contains the RNA. The mature particle contains multiple copies for encoding the structural proteins of the envelope (E), capsid (C) and membrane (M with the precursor prM), and seven non-structural proteins (NS). The genes of the non-structural proteins encode NS1, NS2A and B, NS3 (protease and helicase), NS4B and NS5 (methyl-transferase and RNA polymerase). These proteins orchestrate the production of new viruses once the virus infects a cell.

Humans are the host for the vector of dengue. The bite of infected female mosquitoes can transmit the virus. *Aedes aegypti* lays its eggs, for example, in man-made peridomestic containers. The Asian vector of dengue, *Aedes albopictus*, has become rapidly established in the United States, South America, Africa and Europe. *Ae. aegypti, Ae. albopictus* and *Ae. polynesiensis* have caused dengue outbreaks.

Less common modes of dengue virus transmission occur via blood transfusion, solid organ or bone marrow transplantation and vertical transmission from an infected mother to her child.

Antibody detection

The laboratory diagnosis of dengue infections is based on detecting the virus with molecular methods or detecting antibodies or isolated virus. The choice of the diagnostic method depends on the stage of the disease. For acute infections the virus is found in serum, plasma, blood cells or tissue samples in the first 2-7 days.

Diagnosis by serology (IgM & IgG detection) is performed by Ig capture or direct Ig detection. Commercial tests are available as ELISA, or as strips for rapid tests. For IgM detection, the sensitivities of ELISA tests are in the range 61.5 to 100% and
for IgG detection between 46.3 and 99%, hence, much more sensitive than rapid tests. Most of the time both methods require paired sera to definitively confirm the diagnosis.11 For primary infections the first anti-dengue immunoglobulins (IgM) are detectable in the febrile stage (by day 5 or day 10) and rise to a peak 2 weeks after onset of symptoms.12 IgM can be detectable up to 179 days after onset of symptoms.13

Anti-dengue IgG appears at the end of the first week of illness and rises slowly. For a secondary infection with dengue viruses, which can lead to dengue hemorrhagic fever, it is IgG that arises first and rapidly increases, whereas IgM antibody levels stay lower than in primary infections. One of the pitfalls for detection by serology is the false-negative result for IgM antibodies in secondary infections, and moreover IgM antibodies can cross-react with other flaviviruses (e.g. Japanese encephalitis virus, West Nile virus, Yellow fever virus and St. Louis encephalitis virus).12 Other serological methods for dengue detection are hemagglutination-inhibition (HI), complement fixation (CF), neutralization test (NT), immunoglobulin M (IgM) capture enzyme linked immunosorbent assay (MAC-ELISA), and indirect immunoglobulin G ELISA.14

**Virus isolation**

From 2 to 3 days prior to, and up to 5.1 days after the onset of symptoms, isolation of the dengue virus is also possible.15 For this diagnosis, samples must be collected early in the course of illness (isolation rate 85.3% before day 4 of illness16) and need to be treated promptly after collection. The most sensitive (sensitivity of 71.5 to 84.2%) after an incubation period of 2-7 days, dengue infections may be asymptomatic or, depending on the virulence and other factors, may lead to dengue hemorrhagic fever (DF), or the more severe forms of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Dengue hemorrhagic fever is accompanied by high fever, hemorrhagic phenomena, hepatomegaly and circulatory failure. The pathologic hallmarks are thrombocytopenia, bleeding and plasma/vascular leakage, resulting from increased vascular permeability and abnormal hemostasis. Constitutional symptoms like sore throat and an inflamed pharynx are common. After 2-7 days of fever the disease may deteriorate and progress to the dengue shock syndrome. This is connected with cold and clammy skin, a rapid, weak pulse and restlessness. A patient in shock must be treated with volume-replacement therapy, otherwise the patients dies within 12-24 hours. Complications connected with the shock are metabolic acidosis and severe bleeding from organs. Once the shock is overcome, good prognostic signs are adequate urine output and the return of appetite.1,6 After an infection with one serotype of the dengue virus individuals are immunized for this serotype, but not for the others.
method to isolate the dengue virus is to inoculate mosquitoes in adult or larvae stages. Suitable specimens are serum, plasma, cerebrospinal fluid, peripheral blood leukocytes or tissues. The detection of infection is performed by serotype-specific immunofluorescence in the crushed heads of the mosquitoes. With this method the risk of cross-contamination is very low, but time and effort is very high. 5-20 mosquitoes need to be inoculated per specimen and rearing them is very complex.

Slightly less sensitive but more widely used, is the cultivation of dengue viruses in cell lines, such as AP61 and C6/36. The cell cultures need to be screened by an immunoassay, since most dengue serotypes induce few cytopathic effects (CPE).

The least sensitive (sensitivity of 40.5%) methods are the cultivation in vertebrate cell lines, such as Vero or LLC-MK2, or in suckling mice. In these cell lines the development of plaques can only be identified by passaging.

The intracranial infection of suckling mice leads to illness, paralysis and death within days to 2 weeks.

Isolated viruses in cell-cultures or mouse-brains need to be detected with serotype-specific anti-dengue antibodies. The antibodies are labeled with a second antibody. The advantage of virus isolation is providing a virus isolate for further studies such as sequencing, etc.

Detection of the NS1 antigen has recently become a very popular diagnostic method. In peripheral blood it offers the advantage of a longer diagnostic timeframe compared with virus isolation or RT-PCR. The range of sensitivity of commercially available tests differs from 54.2 to 93.4%. NS1 detection can also be used with other specimens such as tissue samples. In general the method is more suitable for primary than for secondary dengue infections.

Real-time PCR

A rapid, sensitive and specific method for detecting dengue viral genes is reverse transcription-PCR (conventional and real-time using SYBR Green or labeled oligonucleotide probes).

Real-time PCR (Polymerase Chain Reaction) was introduced in the mid-1990s, and shows specific advantages over conventional gel-based PCR. This method allows direct quantification of PCR products in real time.

Real-time PCR amplification detects and quantifies a fluorescent reporter molecule whose signal increases in proportion to the amount of target amplification product generated. Basic methods involve detecting a DNA-binding dye. The dye intercalates between double-stranded DNA formed in the PCR reaction, and when exposed to an excitation source, fluoresces. A laser scanner detects the fluorescence emitted by dye-impregnated DNA strands formed through the PCR amplification. The real-time detection systems include TaqMan or hydrolysis probes.

The combined properties of high sensitivity, specificity, and speed

FTD KITS have a high degree of sensitivity and are used for quick and accurate diagnosis.
has made real-time PCR technology a highly attractive alternative to culture methods, biochemical tests, and microscopy-based methods for diagnosing many infectious diseases. It has become an indispensable tool for state-of-the-art diagnostics of important human pathogens.

To increase the diagnostic capacity of PCR, multiplex PCR (vs. singleplex) has been developed. Multiplex RT-PCR allows the detection of more than one target in one reaction. The technique is useful for simultaneous identification of viruses, bacteria, fungi, and/or parasites, thus saving time, effort, and money. Multiplex PCR has become a rapid and convenient screening assay in both clinical and research laboratories, as several target sequences can be amplified by including more than one pair of primers in the same reaction. Since molecular-based testing for multiple pathogens in a large number of humans is expensive, multiplexing offers a more cost-effective solution.

The disadvantages of multiplex real-time PCR is the risk of false-positive or false-negative results if the assay is not optimized carefully, and the possibility that the clinician may not be satisfied with the selection of different pathogens in the multiplex.

The advantages of real-time PCR as a diagnostic tool are that the assay results can be determined within one day, so results may return more quickly than with culture assays. Moreover real-time detection allows the accurate quantification of the number of RNA or DNA copies present in the test sample, thereby providing important information on the extent of viral involvement within a test sample.

**Fast-track kits**

Commercial test kits are available for the diagnosis of dengue infection using real-time PCR. Current tests are between 48.4 to 98.5% sensitive, and more than 95% specific. The start of the diagnostic procedure is the extraction of nucleic acids. To ensure the proper processing of samples without contamination, negative and internal controls are included during extraction. The tests are for use with extracted nucleic acids from whole blood or urine of human origin.

Fast-track Diagnostics recommends the use of thermo cyclers providing the 4 fluorescence channels FAM, YAK/HEX/VIC, ROX, and CY5/ATTO647. All of the FTD kits are validated on different cyclers, e.g. Applied Biosystems® 7500 / 7500 fast (life technologies™), CFX96™ (Bio-Rad), LightCycler 480® (Roche), Rotor-Gene® Q / 3000 / 6000 (Qiagen) or SmartCycler® (Cepheid). For the setup of the

**Fast-track Diagnostics (FTD)** is a Luxembourg-based molecular diagnostic company focused on developing and commercializing real-time PCR (RT-PCR) assays to simultaneously detect a wide range of viruses, bacteria, fungi, and parasites in one experiment. For over five years, FTD has been developing new assays and participating in international projects (e.g. the Bill and Melinda Gates Foundation funded PERCH project). Since October 2011, FTD has been ISO 13485 certified and most of the tests are CE certified.

FTD’s kits are based on disease categories – the syndromic approach. The whole range of FTD assays can be run using the same protocols, on a wide range of cyclers and polymerase enzymes. Each assay uses the same dyes, resulting in an efficient workflow without requiring modification between runs and assays, and without additional training of personnel.

Since late 2012, FTD has offered a new disease category called tropical fever. This range of kits contains tests that simultaneously detect dengue and chikungunya, and differentiate between the 4 dengue virus types, or determine the cause of fever by detecting dengue, chikungunya, West Nile, salmonella, plasmodium, leptospira and rickettsia.

**Further information:** www.fast-trackdiagnostics.com
procedure with a universal cycling program for all RNA and DNA pathogens, FTD recommends a one-step RT-PCR kit that enables the simultaneous detection of RNA and DNA pathogens. This enzyme combines a reverse transcriptase with a Taq polymerase.

Detection of the pathogens takes place via specific primers of conserved regions and a dual-labeled molecular probe specific for each pathogen within the multiplex PCR. The presence of specific sequences in the reaction is detected by an increase in the fluorescence observed from the relevant dual-labeled probe, and is reported as a cycle threshold value (Ct) by the real-time thermocycler.

The FTD-Dengue/Chik uses Brome mosaic virus (BMV) as the internal control, which is introduced into each sample including the negative control at the lysis buffer stage of the extraction process.

For correct interpretation of the results the negative control should be below the threshold. If there is potential contamination (appearance of a curve), results obtained are not interpretable and the whole run (including extraction) has to be repeated. The positive and internal controls must show a positive (i.e. exponential) amplification trace with a Ct below 33. If the internal control falls out of this range, this points to a purification problem. After successful extraction the test delivers the result within two hours.

**Distinguishing dengue and chikungunya**

Chikungunya is a mosquito-borne, single-stranded RNA alphavirus, belonging to the Togaviridae, and causes fever, joint pain, headache, and prostration.

In regions where dengue and chikungunya are endemic, they must be differentiated to avoid misdiagnoses. Differentiation by observation of constitutional symptoms is not possible because both diseases are frequently associated with an inflamed pharynx, vomiting, constipation, headache, generalized lymphadenopathy, and conjunctivitis. With the same sensitivity as for the single detection of both pathogens, the FTD-Dengue/Chik kit is suitable to distinguish very fast between the viruses.

To estimate the high risk of complications occurring in the case of a subsequent infection with another, different serotype, and to react quickly, it is important to distinguish between the dengue virus types 1-4. Only during the first few months after a dengue infection are people cross-protected against the other dengue serotypes. After the first dengue infection patients have an increased life-long risk of hemorrhagic fever or shock syndrome with a second dengue infection.

The difficulties associated with conventional methods of serotyping dengue-infected samples can easily be solved by using the FTD-Dengue differentiation kit. The principle of this kit is nearly the same as for FTD-Dengue/Chik. With this multiplex kit it is possible to differentiate between the four dengue virus types in one tube. The same extracted material used for the FTD-Dengue/Chik should be used again because no internal control is included. Both FTD kits for the detection and differentiation of the dengue virus have a minimum detection limit of 0.1 target copies/μl and show 100% sensitivity and specificity.

**CONCLUSION**

It is essential to diagnose dengue as soon as possible, and assess the risks of DHF or DSS developing to provide proper treatment. It is therefore important to distinguish between the different dengue virus types as well as chikungunya virus. FTD multiplex real-time PCR kits provide clinicians with a rapid, reliable and accurate diagnostic solution. The technique is simple but dependable, and due to the high sensitivity and specificity there is no need to wait for the results of conventional diagnostic methods.
mosquito-borne viruses such as the four dengue serotypes (DENV 1-4), yellow fever (YFV) and chikungunya (CHIKV) are responsible for millions of human infections and significant morbidity and mortality each year. Also the geographic range of these viruses in particular is expanding. An article by Nathan on CHIKV (see PHJ No. 18) discussed the significance of the 2005-2006 outbreaks in the Indian Ocean islands. Targeting the vector has been used to reduce the incidence of arboviral diseases since the 1870’s. With the spread of insecticide resistance in mosquito populations (see PHJ No. 18), control of vector-borne diseases really requires an integrated strategy that targets multiple components of the transmission cycle.

Vaccines and vector control

Understanding the transmission cycle is vital and an important difference between YFV and DENV/CHIKV is that unlike YFV, neither DENV nor CHIKV have a significant sylvatic/zoonotic component to their transmission cycles. This actually means that compared with YFV, a vaccine-based approach targeted at protecting humans from DENV/CHIKV infections should be more effective at breaking the transmission cycle because, unlike YFV, neither of these viruses can be sustained in the absence of human infections.

Several promising vaccine candidates for DENV are in advanced stages of evaluation; however, the process is long, rigorous and highly regulated, with international oversight by
multiple agencies. In the absence of approved antiviral agents or licensed vaccines, control of DENV and CHIKV has focused on control of the mosquito vector using adulticides or larvicides, or through source (i.e. breeding site) reduction. The potential use of insecticide-treated bednets (ITNs) for dengue control was discussed in Public Health Journal No. 22.

Genetically modifying mosquitoes

The possibility of using genetic manipulation to control mosquito vector populations to manage disease spread was discussed long before the technology to accomplish this goal was available. Early successes in producing genetically modified mosquitoes, some twenty years later, were very labor intensive with no real reproducibility. The first successful transformation of a mosquito was of *Anopheles gambiae* followed by transformation of *Aedes triseriatus*, then *Ae. aegypti*. At this time the process relied on microinjection of plasmid DNA into mosquito embryos, but there was no specific method to ensure integration of the DNA into the mosquito genome.

The discovery and development of transposable elements has enabled the genetic manipulation of several different species of mosquito vectors with relative ease, and there are now facilities that will create genetically engineered mosquitoes for customers, such as the University of Maryland Institute for Bio-science and Biotechnology Research “Insect Transformation Facility Services.”

The *piggyBac* transposon has proved especially useful to transform mosquitoes such as *Ae. aegypti* and *An. gambiae*, but several others elements have also been used. New markers have been applied for non-lethal screening of larvae and adults for transformation, including the cinnabar gene for eye pigmentation and green fluorescent protein. Tissue specific mosquito promoters to drive gene expression have been characterized, for example carboxypeptidase. The utility of a meiotic drive approach has been discussed for population replacement but has not been developed for mosquitoes.

Although mechanisms that might confer viral resistance have been identified, for example expression of single chain antibodies and “antisense” strategies or RNA interference (RNAi) that can essentially prevent DENV replication in mosquitoes, it is remarkable that after over 100 years of study, the exact mosquito genetic basis that determines whether a mosquito is susceptible or resistant to infection is unknown. Since the susceptibility of mosquitoes to DENV or CHIKV is a non-linear/discrete phenomenon (i.e. not all or nothing, but rather ranging from 0 to 100%), then it is likely that susceptibility is influenced by multiple genes, polymorphisms within the genes, and also by environmental factors.

How transgenic mosquitoes could control dengue and chikungunya

In terms of the process of developing and using genetically modified mosquitoes, three phases have been envisioned: technology exploration, field testing, and full implementation as a routine control measure. As a prerequisite, it is important to conduct fundamental studies on vector transmission of arboviruses, and then to study vector capacity traits such as host preference, longevity, density, and other characteristics.

As mentioned above, we still do not fully understand mosquito-virus relationships, so such knowledge is critical if the promise of controlling mosquito-borne diseases via genetically reducing vector competence of mosquitoes is to be fulfilled.
Obviously all activities must be coordinated so that proper regulation, community engagement and investment are in place as the technology proceeds. Regulatory and community engagement should be key components for planning the application of engineered vectors for disease control, and these are discussed below.

Vector genetic-based approaches that might be used to control DENV and CHIKV may be broadly categorized into two types: direct and indirect. A variety of mechanisms may be used within each approach, including knocking out an endogenous gene to confer resistance; introducing a sequence that prevents infection, dissemination and/or transmission or an inducible fatality gene; Wolbachia-induced cytoplasmic incompatibility or lifespan reduction; or radiation-induced sterilization.

Although not commonly regarded as genetically altered insects, Braig and Yan stated that “there is a very fine and, at the end, a totally artificial and unjustifiable line between transgenic organisms and genetically altered or genetically modified organisms.” Any method that artificially causes mutations was considered to be genetic manipulation; therefore, mosquitoes that have been irradiated to induce sterility could also be categorized as transgenic like those which have been transformed, and are discussed below (SIT). It should be realized, that for the general public, the term genetically modified/engineered, may be viewed quite differently to sterile insects.

**Direct approaches**

The suggestion that vector-borne diseases might be controlled better by reducing the vectors’ ability to transmit pathogens rather than by reducing vector populations has been embraced by many, and great progress has been made towards testing the feasibility of this approach. A direct, or virus-specific, approach might involve the use of mosquitoes that were genetically less able to transmit the target virus, perhaps because they were less susceptible to infection due to a variety of mechanisms.

In a human DENV infection, blood viremia is relatively brief, increasing then decreasing during a 4-5 day span. If the peak viremia titer is below the threshold needed to infect a mosquito, then the mosquito is functionally refractory. A highly susceptible mosquito is basically one for which the threshold of infection is low, whereas a refractory mosquito is one that cannot be infected, either because it is absolutely resistant to infection, perhaps lacking essential receptors in the midgut, or because the threshold for infection is much higher than would be reached in nature. As an example, one of the significant safety components of the ChimeriVax vaccine platform (in development and testing for numerous arboviruses including DENV) is that while vaccinated humans can become viremic, the titers reached are below the threshold that would be required to infect mosquitoes. Therefore the vaccine virus would never be transmitted beyond the vaccinated individual. Additionally, the vaccine viruses are relatively non-infectious to mosquitoes and rarely if ever transmissible.

**Breaking the transmission cycle**

In contrast to knocking out endogenous (i.e. mosquito) genes/sequences to confer resistance (lack of susceptibility), an alternative approach would be to introduce a sequence that prevents completion of any critical step between uptake of a viremic bloodmeal by a mosquito and transmission of virus by that mosquito to the next susceptible individual. From the perspective of breaking the DENV or CHIKV transmission cycle, one does not necessarily need to have a mosquito that cannot be infected. A mosquito that can be infected but cannot transmit the virus is sufficient. Transmission can be prevented by lack of adequate virus accumulating in the salivary glands, or by the mosquito simply dying before this titer in the salivary glands is achieved (the indirect approach, discussed below).

The likely existence of more than one “susceptibility gene” which may or may not be different for different mosquito populations and the fact that the
The genetic influence of susceptibility is not necessarily a general trait but can also be virus-specific (i.e., *Ae. aegypti* engineered for DENV resistance may be only partially resistant or fully susceptible to CHIKV) could spell trouble for the direct approaches. Another problematic possibility is that any genetically-conferred resistance trait could be lost over multiple generations, or that a fitness cost associated with the genetic modifications would drive engineered mosquitoes out of the population. Clearly, testing of any candidates for genetic engineering would need to include robust stability and fitness evaluations.

### Indirect approaches

More broadly applicable (and therefore, presumably more efficient) approaches to controlling DENV and CHIKV using transgenic mosquitoes would be “indirect” or non virus-specific. These approaches would aim to reduce the total adult population or just the percentage of the adult population able to transmit DENV or CHIKV. Historically this has been accomplished by the use of insecticides. Insecticide use and insecticide resistance have been the subject of multiple articles in Bayer’s *Public Health Journal*.

The multi-country use of insecticides to kill *Ae. aegypti* in the Americas\(^8, 42\) was associated with a spectacular success that effectively eliminated dengue, and via the incidental reduction of Anopheles populations, also malaria. Of course when these control programs were relaxed, *Ae. aegypti* and DENV reinvaded all of the former territories and *Ae. aegypti* expanded its geographic range by the 1990s to become more widely distributed than ever before\(^17, 18\).

#### Sterile insect technique (SIT)

Irradiation techniques for sterilizing large numbers of insects that are then released into the wild have been tried for other species, for example *Culex* in India to control filaria. Although no longer used for mosquito control, the sterile insect technique (SIT) based on radiation or chemical sterilization, is currently used to control a variety of agricultural pests in the United States and elsewhere, including the New World screwworm *Cochliomyia hominivorax*, the Mediterranean fruitfly (*medfly*) *Ceratitis capitata*, and the pink bollworm *Pectinophora gossypiella*.

The basis of the SIT strategy is that by releasing large numbers of sterile male insects, wild type females are more likely to mate with sterile males than with fertile ones. Since no progeny are produced, the species is driven to extinction. A key to this strategy is that one must release a sufficient number of sterile males to overwhelm the numbers of wild type males. Another key element is that females of the target species only mate once. As populations of insects are eliminated from the periphery (e.g., the northern part) of their range, the release program is moved so that slowly the distribution is progressively reduced.

Using SIT, the New World screwworm has been eliminated from the US and many other regions. Although the effort is huge and the cost is enormous (approximately 45 million flies are reared a week), the screwworm is an important pest of domestic animals and causes a significant impact on agricultural trade and revenue. Presumably, the cost of controlling these flies far exceeds the economic consequence that would be endured if they were not controlled.\(^14\) SIT has been used in conjunction with insecticides, physical barriers, cultural control, regulatory control, and quarantine for permanent control of the medfly.\(^48\)

Control of the pink bollworm has been regarded as one of the most successful applications of SIT\(^51\) and has been described as the key precedent for the field use of genetically modified insects.\(^2\)

#### Releasing insects carrying a lethal gene

In the last few years, a new technique has been developed that has been described as a modern adaptation of SIT, the Release of Insects Carrying a Dominant Lethal gene (RIDL).\(^32, 40\) A transposon is inserted into the
mosquito genome, which necessitates the inclusion of tetracycline in the diet for survival. Since the gene is dominant and heritable, the progeny of engineered males and wild-type females also require tetracycline to survive. In practical terms this is equivalent to releasing sterile males because when they mate with females, effectively no offspring are produced (Figure 1).

A male-only release strategy is necessary since released females would still be capable of viral transmission; males must either be mechanically sorted from females before release or an alternative line in which only males are produced must be used (Figure 2). The first release of RIDL male mosquitoes, at a ratio of an estimated 10 engineered males to one wild-type female on Grand Cayman, resulted in an 80% reduction of the local mosquito population (Figure 3). Although this was successful on a small scale, it remains to be seen whether this is practical on a large scale. Given the way that *Ae. aegypti* rapidly reinvaded areas from which it had been eradicated in the Americas after control was relaxed, it may be necessary to release mosquitoes for years, perhaps indefinitely, for RIDL to succeed.

**Wolbachia-infected mosquitoes**

Another approach involves the use of the obligate, intracellular rickettsial symbionts *Wolbachia*. This could be used to reduce the percentage of the adult mosquito population that could transmit DENV or CHIKV through a phenomenon known as cytoplasmic incompatibility. In a mixed population of *Wolbachia*-infected and uninfected mosquitoes, a cross between an infected male and an uninfected female is incompatible in that embryonic death occurs and viable offspring are not produced (Figure 4). Uninfected mosquitoes therefore become less common, and if infected mosquitoes have a shorter lifespan, i.e. die before they can transmit the virus, or...
are relatively poor virus vectors, then viral transmission efficiency may be reduced.

Recently it has been discovered that one strain of Wolbachia not only reduces the lifespan of Ae. aegypti by up to 50% but also confers resistance to infection with DENV and CHIKV\cite{6,33} and YFV\cite{49}, perhaps through activation of the Toll pathway.\cite{39}

Release of Wolbachia-infected mosquitoes in two towns in Northern Queensland resulted in 90% and 100% infection of trapped mosquitoes four months later, but these percentages declined to 81% and 95% two weeks later; so much like the other techniques mentioned above, it remains to be seen whether Wolbachia would be effective in reducing DENV and CHIKV transmission in the long-term in an endemic region over a wide area.

**Environmental, cultural, and economical concerns**

As far as direct effects on the environment are concerned, there are no obvious ecological impacts of the methods discussed above that are different from insecticide control. In fact many scientists believe that eliminating mosquitoes would not have great ecological consequences. The development and release of transgenic organisms, especially those that have a substantial impact on human morbidity and mortality, is an emotive and sensitive issue, and there are well-documented statements regarding the importance of community involvement and supposed requirements for permitting prior to release.

As early as 1994, Hoy included in her book “Insect Molecular Genetics: An Introduction to Principles and Applications” a discussion of the importance of evaluating risks associated with the release of transgenic arthropods into the environment. Another book chapter titled “Ecological and community considerations in engineering
arthropods to suppress vector-borne disease," asserted that the large-scale release of genetically engineered organisms should not be permitted unless the following requirements are met: the released organisms do not annoy local residents any more than any ambient vector organisms do; the release results in no increase in abundance of hematophagous arthropods requires no reduction in ongoing health-promoting activities, and does not compromise future interventions against the target disease; the force of transmission of microbes other than the target pathogen would not increase; and any improved state of health of people living in the release area is sustainable.

The testing phase must include experimentation in the laboratory and contained or “caged” field trials before any large-scale release of transgenic organisms is permitted. This includes necessary phenotypic evaluations of the transgenic strain with testing for adverse effects on target and non-target species. Selection and characterization of a field site for testing should include scientific, regulatory, and community engagement considerations, and community and public engagement should go hand-in-hand with evaluation of long-term effects of release and sustainability following an open field evaluation. To its credit, the Gates Foundation that has strongly supported the development of genetically engineered mosquitoes for disease control has taken ethical, social and cultural issues very seriously. With the direct approaches, even if the magic genes/sequences for knockout or insertion into the genome are identified, the possibility that there would be fitness costs associated with any modification to the mosquito genome is a very real one, which could render an engineered mosquito population unable to become established in the wild. The two approaches currently showing the most promise for DENV and CHIKV control are RIDL and use of Wolbachia.

Can transgenic mosquitoes really control dengue and chikungunya?

Given the substantial hurdles that exist to the development, implementation, and sustainability of transgenic mosquito-based DENV and CHIKV control, one must ask the question, is it reasonable to expect this approach to be successful? Research, development, testing and efficacy evaluation of candidates for transgenic mosquito-based virus control has been, and will continue to be, an enormously expensive undertaking. Previous successes in insect elimination using transgenic insects, such as the screwworm, have had enormous measurable economic and political benefits, and obviously the elimination of DENV and CHIKV would be highly significant in terms of humanitarian and economic benefits.

Taking cost out of the equation leaves the technical and logistical challenges of spreading and establishing mosquitoes with reduced vector competence into wild populations. With the direct approaches, even if the magic genes/sequences for knockout or insertion into the genome are identified, the possibility that there would be fitness costs associated with any modification to the mosquito genome is a very real one, which could render an engineered mosquito population unable to become established in the wild. The two approaches currently showing the most promise for DENV and CHIKV control are RIDL and use of Wolbachia.
human community\textsuperscript{2}, the question of who will pay and how long that effort will be sustained remains.

Furthermore, if \textit{Ae. aegypti} is eliminated from an area, there is the possibility that another mosquito species such as \textit{Ae. albopictus} would take its place. Although \textit{Ae. albopictus} is thought to play a minor role in DENV transmission\textsuperscript{26}, it is a very efficient vector of CHIKV.\textsuperscript{46, 47}

In contrast to RIDL, the \textit{Wolbachia} approach has the advantage of being a potentially self-perpetuating system because \textit{Wolbachia} could drive itself through \textit{Ae. aegypti} populations. However, although it does not theoretically require constant releases, a large number of mosquitoes were needed to establish the populations in the two Queensland field trials, and the infected populations were declining when last tested. Clearly, more work needs to be done to determine the long-term sustainability of this approach.

**Future implications**

Yellow fever provides a good example of the need for multiple approaches to disease control. Despite the existence of the very effective 17D vaccine, YFV still causes hundreds of thousands of infections and tens of thousands of deaths each year. The existence of a YFV sylvatic/jungle component compromises the effectiveness of vaccine-based disruption of the transmission cycle because of susceptible wild primate hosts. By necessity, mosquito control is needed to augment vaccination campaigns.

To have any effect on the incidence of dengue or chikungunya, the RIDL technology depends on continued release of mosquitoes. In contrast, the \textit{Wolbachia} based approach may be self-sustaining but if resistance to arboviral infection is lost, one may simply have arbovirus-susceptible \textit{Wolbachia}-infected populations replacing susceptible uninfected populations. If the infected mosquitoes have a reduced lifespan, this may have an effect. It is unclear if being infected with, for example the wMel strain of \textit{Wolbachia} would compromise future releases of more effective strains of \textit{Wolbachia} if identified.

In 2011, Osteria and Gostin recommended that “a new treaty should establish an international process for rigorous examination of the scientific evidence, ethical values, and dispassionate review before genetically or biologically modified arthropod vectors are released into the natural environment”. Remarkably, despite years of discussion, there are still no regulations that have been universally agreed upon for development and release of transgenic mosquitoes, and yet both RIDL and \textit{Wolbachia} infected mosquitoes have already been released into nature. Given that these mosquitoes respect no borders, it seems almost irrelevant that permission is obtained from one country since the mosquitoes may invade adjacent ones that have not been consulted. Any international treaty developed at this stage, seems to be as we say in England “shutting the stable door after the horse has bolted”.

**CONCLUSION**

It seems unlikely that using genetically modified mosquitoes alone will accomplish control of DENV and CHIKV. Rather, modified mosquitoes might be used as part of a coordinated effort that employs synergistic efforts to include widespread vaccination of the human population and conventional vector control. Vaccination would not only directly protect the human population but also help break the viral transmission cycle by reducing the number of infected hosts from which mosquitoes may become infected. However, until effective DENV and CHIKV vaccines are developed and approved for human use, the mosquito component of the transmission cycle is the only viable target, and new genetic strategies are worth considering.

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Dengue fever: targeted biological vector control

Changing times, changing minds

By introducing a genetic modification into male *Aedes aegypti*, Oxitec has developed a technique where offspring die before maturing to adult mosquitoes. Modified males efficiently seek out and breed with females, reducing mosquito populations to minimal levels. Combined with all the other tools of integrated vector management, this offers the chance to provide superior control and look toward elimination of this primary vector in urban areas.

Dengue is only spread through the bite of an infected female mosquito. With no other solution vector control must therefore form the main line of defense against the virus. Current mosquito control techniques revolve around clearance of breeding sites, treatment of water with larvicides and adulticides for adult mosquitoes. However treatment of all standing water in a town, every container, every blocked drain or concave roof, is clearly a major challenge.

For an optimum result the key is one integration; combining in an optimum way not only a series of different interventions but also bringing together the efforts of a well planned public health program with the support and compliance of the local community.

Experience from even the best-funded mosquito control budgets around the world shows that with current technologies a good result is to control the growth in numbers of the insect from year to year rather than eliminate the threat. Dengue programs therefore tend to be about management and containment with a strong focus on community-based activities to control the proliferation of breeding sites.
**Oxitec’s new solution**

However a new approach is now being evaluated in several countries around the world with Brazil being the most advanced. There, following earlier trials, the evaluation is now taking place in a town of circa 50,000 people. The Oxitec concept is derived from the use in agriculture of releasing sterile insects (a practice called the Sterile Insect Technique or SIT).

In agricultural SIT programs, fruit flies or moths are reared in huge numbers and then sterilized with radiation. The radiation causes mutations that render the male effectively unable to reproduce. Insects are then released into the environment with the objective that sterile males will mate with fertile females, whose offspring then die. SIT programs tend to target area-wide elimination of a pest or maintaining area-wide pest-free zones. These have been very effective.

SIT works well with great effect for some species of insect, but not for others. Not all species can tolerate the radiation dose required to provide a sterilization effect without becoming so unfit as to lose their ability to compete against wild males for females to mate with. For example, it has not been successful with mosquitoes.

**Modified male mosquitoes**

But now, using genetics, the same end result can be achieved across a wider variety of insect species with high precision and increased efficiency. Not only can the approach now be applied to new species, but using genetics avoids the harsh fitness penalties associated with radiation, meaning that the approach is more cost efficient.

Oxitec has pioneered this new method and has developed insect strains for both agriculture and vector control. Oxitec insects carry a genetic element that causes the death of the next generation. The first of the Oxitec strains to be evaluated around the world in open release trials is OX513A; a strain of modified *Aedes aegypti*.

For mosquitoes, two very simple features of biology are important to this method of control. Firstly male mosquitoes do not bite or spread disease, only females do. So the release of males does not create a health issue. Secondly, males are very good at finding females; far better, in fact, than any human. And a mosquito, unlike a human, does not have to request permission to enter private property! The males will seek the females wherever they reside.

**Reducing populations to minimal levels**

Oxitec release male *Ae. aegypti* mosquitoes, which then seek out and mate with their wild counterparts. The offspring will die prior to becoming functioning adults. In open release trials in Cayman, Oxitec have shown that this approach can suppress a local *Ae. aegypti* population by over 80% (compared to the neighboring control area)\(^1,2\) and despite the population being at

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"Today, dengue ranks as the most important mosquito-borne viral disease in the world. Everywhere the human and economic costs are staggering".

Margaret Chan, Director General, WHO

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The author:

**HADYN PARRY**

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its seasonal peak, in as few as five months. In the next trial in Brazil suppression of 85% was achieved and this was then surpassed by the most recent trial that was in a more isolated site.

In such scientific trials, where treated sites border on an untreated area one should expect some re-introduction of *Ae. aegypti* from neighboring areas, hence total elimination is unlikely. But in a structured control program, or in areas of reasonable isolation, or indeed where complementary control measures are implemented, the *Ae. aegypti* population can certainly be reduced to minimal levels.

**Highly targeted biological control**

Sustainability and environmental aspects dominate the thinking when evaluating new tools in vector control. In these respects the credentials of the new approach are very strong. While some interventions are broad spectrum, Oxitec insects affect only their own species. *Ae. aegypti* only mate with *Ae. aegypti*. On release, the mosquitoes do not travel far, meaning that control is limited to the release area. Inability to reproduce lies at the very heart of the control, and the mosquitoes themselves die out in days, so there is no persistence in the environment.

Furthermore, the *Ae. aegypti* mosquito is an invasive species in most countries. Its global spread has been facilitated by humans in recent years through the movement of people and freight. Its elimination or removal is therefore restorative in terms of the local native ecosystem.

**Putting into practice**

There are two main challenges in controlling this urban mosquito. The first is to reduce the population of the vector, and the second is to maintain that level of control once it has been reached. In both cases this needs to be achieved in the most cost effective way possible. Budgets are always tight. To achieve control in an area it is important that wild females mate with the Oxitec ‘sterile’ males. The greater the number of sterile males released relative to their wild counterparts, the greater the frequency and points of release, the faster the population will decline.

Outnumbering the local wild males can be achieved in a number of ways; for example using early season release when the wild mosquito population is low means that less sterile males are required and that the seasonal surge in mosquito population that would otherwise occur in the rainy season is avoided. Equally, parallel use of larvicides or an insecticidal pre-treatment knockdown are other options. Once control has been achieved one might use a steady low-level release of the sterile mosquitoes to prevent re-infestation, or use a combination of approaches such as low-level release in the most likely areas of reintroduction and other interventions for “hot spots”. Alternatively good quality monitoring could be used as the trigger for continued intervention.
Integrated vector management

As time progresses different operational practices of integrating Oxitec’s biological control method with existing conventional tools to emerge are expected, in the same way that the use of beneficial insects are now used in integrated pest management programs in agriculture. Some interventions, for example space spraying, can be highly effective in reducing large populations in mosquitoes to a lower level. But as the level decreases there are diminishing returns.

However with the Oxitec approach, since males will activity seek out the females, this reduction in cost efficacy does not apply. A male *Ae. aegypti* can fly up to circa 200 meters to find a mate, and as the numbers of females decline the control becomes more targeted and efficient. Different interventions have different strengths, but their combination is likely to prove the most cost effective.

Fluorescently tagged for monitoring

Under any of these options, and as with pest management in any sphere, monitoring is critical. A key challenge in any current vector control program is actually establishing the baseline level of the *Ae. aegypti* population and the ability to monitor its decline – so a program of control can be adapted overall or locally. In this respect monitoring is greatly facilitated because the Oxitec mosquitoes themselves are identifiable. They have a protein marker that is visual at the larval stage using specific fluorescence. In an operating program releasing a set number of males and seeing the change in the male-female ratio will help to establish the overall population level.

Collecting larvae from ovitraps and identifying the percentage of the sample containing fluorescence on a regular basis will, together with the overall number present, help establish the success of the program. The fluorescence provides a simple and usable tool to monitor the program while it is in operation, to flex the interventions and to predict the population decline.

**CONCLUSION**

The *Aedes aegypti* control challenge is immense. Its rapid introduction into new geographies, its adaptation into the urban environment and its relatively dispersed presence in towns and cities mean that interventions by health authorities in many countries focus on public education and behavior on the one hand and ‘fire fighting’ on the other. A mindset of containment or management can result. But now, by integrating the new approach of Oxitec mosquitoes with current tools, the mindset can change to one of actually targeting reduction in the population of this invasive species in towns and cities to such a degree as to have a highly beneficial impact in disease prevention.

More

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Article on the enclosed Public Health CD-ROM

**Sources**


3. McKemey et al., in prep
On August 23, 2012 a plastic surgeon from Mulago hospital in Uganda was quoted as saying that the majority of burn cases he handles result from mosquito nets catching fire, and that nets are the worst fire agent that can burn children. In the same article a situation was described where a paraffin lantern set alight to the mosquito net over the bed of a child, subsequently causing severe burns to the child’s legs, arms and other parts of her body. Statistics, quoted in the same article (from the Uganda Burns and Plastic Surgery Institute at Mulago Hospital) show that Uganda averages 80,000 burn victims every year.

Fire safety is an important aspect that must be taken into account in the development of long-lasting mosquito nets. Many burn accidents or even fatalities are reported to be due to bednets catching fire. Less serious damage such as burn holes can greatly reduce the effective lifespan of a net. It is vitally important to use better flammability tests to accurately assess the risks. Bayer Environmental Science has been investigating this while developing its LifeNet mosquito nets.

The Program of Human Biology at Stanford University publishes a periodical online Newsletter highlighting some of its activities; the Fall 2010 edition contained a feature on the work of a student interested in understanding why people did not use mosquito nets that they had received for free. A survey of 332 households in 12 rural villages in Uganda had revealed the surprising result that the fear of bednet flammability was one of the most important reasons why people did not use mos-quito nets that they had received for free. Mothers are afraid to let their children sleep under the nets because open flames are the primary source of light and nets are regarded as a fire hazard. This was a more commonly reported reason for not using the bednet than any lack of ventilation under the net, roughness of the fabric, or other expected responses.

In the BBC News on August 25, 2009 there was a tragic report from Tanzania about a dormitory fire which killed 12 girls. This fire was attributed to a mosquito net catching alight from contact with a candle which one of the girls was using to read by.

The digital edition of the Kenyan Standard from Wednesday December 19, 2012, reported that a baby was burnt to death after one of the siblings had lit a tin lamp whose flame then
caught a mosquito net; the fire then spread to other parts of the grass-thatched house.

Resurge International (formerly Interplast) is a California-based NGO that specializes in reconstructive surgery in developing countries. For 39 years this organization has worked to reduce physical limitations and return people to work or school through surgery. Burns victims comprise half of the surgeries performed by this organization. Their burns factsheet\(^5\) (“The forgotten global health crisis of burns”) refers to “highly flammable mosquito nets as also being responsible for disabling burns in developing countries”.

The University of Toronto, Office of International Surgery\(^6\) highlights the development of public service information (including a powerful video) about burn prevention, targeted primarily for Cambodia, where hospital records indicate burns from mosquito nets catching fire being a common risk.

All these references to the risks associated with the contact between mosquito nets and fire were uncovered with a recent (and basic) Internet search. The multiple general references from separate organizations suggest that these few reports may be just the tip of the iceberg.

Clearly contact of a mosquito net with a flame is a purely accidental occurrence that is to be avoided through proper use, but this does not change the fact that these accidents apparently keep happening.

No documented statistics are available on how frequently this phenomenon occurs, but perhaps some insight can be gleaned from studies that indicate how frequently nets are recorded as being damaged by fire (i.e. burn holes). Some recent studies on LN durability are now recording the causes of hole formation and these results illustrate the potential influence of flammability of net materials on the overall durability of the nets.

In a paper published in February 2013 in the Malaria Journal 12:467\(^7\) (Physical condition and maintenance of mosquito bednets in Kwale Country, coastal Kenya), the following statements were made: “Animals (goats, sheep, cattle, rodents, cats), fire from various sources (oil lamps, sparks from cooking, candles) and snagging on the bed frame are some of the known causes of holes in bednets,” and “...a high proportion of the holes were also caused by fire, mostly in single-roomed houses where the room serves as both kitchen and bedroom. Another source of fire are the tin lamps, that are used by >70% of the families.”

During the Roll Back Malaria Working Group Meetings in February 2012 (presentations published on the RBM website)\(^8\) references were made to a study conducted in western Kenya. In this study 47% of the nets surveyed had burn holes within the first year.

In a study from 2004 of Olyset™ insecticide-treated nets distributed seven years previously in Tanzania\(^9\) it was noted that several nets had lost their original light blue color and had turned grey to almost black due to the fumes from the fires inside homes, and also “many holes clearly resulted from burns rather than tears”.

Where does all this leave us?

It is already established that all labels for mosquito nets should contain wording recommending that the net should be kept away from fire or naked flames. However, the bag which contains the instructions for use is usually discarded quickly after
the first use of the net; the instructions on the tags sown into the seam are hard to read and can fade after long-term use and in some cases the user may not be able to read the label anyway. Combine that with a heavy reliance on paraffin or kerosene lamps and candles as primary sources of lighting in many parts of the world where mosquito nets need to be used and it is no wonder that these accidents keep occurring.

Other efforts such as behavioral change, communication and education during the distribution of nets can clearly play a role in reducing the incidence of damage to, and risk of injury from accidental contact of fire with mosquito nets. But what additional efforts can be made on the side of industry to try to reduce this problem?

It is, of course, self-evident that fire safety is an important aspect that must be taken into account in the development of long-lasting mosquito nets. This is why, in accordance with the WHO specifications for netting materials and mosquito nets (2005)\textsuperscript{10}, all manufacturers of long-lasting insecticide-treated mosquito nets are required to determine the flammability of their nets according to the standard test 16 CFR Part 1610.

However, the propensity of some net materials to burn more readily than others is not something that the current testing requirement (16 CFR Part 1610 test methodology) can easily show. This test, which dates back to the 1970s, was established by the US Consumer Products Safety Commission as part of its requirements for clothing textiles, and allows a piece of fabric material to be classified into one of three broad classes of flammability:

- **Class 1**
  Normal flammability

- **Class 2**
  Intermediate flammability

- **Class 3**
  Rapid or intense burning

This testing process is designed primarily with safety outcomes in mind. The test procedure requires that a 16 mm flame impinge on a fabric sample mounted at a 45 degree angle for 1 second. The sample is allowed to burn its full length, or until a stop thread is broken, a distance of 127 mm. The results from several samples are averaged and a Class designation is made based on the flammability performance and surface characteristics of the sample. If the average time taken for the full length of this piece of fabric to burn is greater than or equal to 3.5 seconds then the fabric is considered to be Class 1; if it consistently burns more quickly than this, then it is considered to be Class 3.

This test therefore classifies materials which will maintain a steady flame into a general group called “Normal Flammability” but it is also clear that this “normal flammability” encompasses material that will still sustain a steady flame for a certain period of time.

The flaws in this test are perhaps best illustrated in the graph shown below:

### Burning behavior of 10 samples for 3 fabric types

<table>
<thead>
<tr>
<th>Fabric Type</th>
<th>10</th>
<th>0</th>
<th>5</th>
<th>5</th>
<th>3</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polypropylene LifeNet (deltamethrin incorporated)</td>
<td>10</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Polyester (deltamethrin coated)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyethylene (permethrin incorporated)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Lessons learnt in the development of LifeNet?

Prompted by the on-the-ground insights of the Bayer Vector Control team in South Africa, it was decided to investigate the issue of net flammability a little further. This not only started to reveal the scope of the issue in terms of frequency of these accidents and their associated injuries, but it also provided insights into a range of other testing methods which are available to show differences in flammability properties between different textile fabrics. Given that bed nets can be considered as textile fixtures within a building and some of the incidents described are more closely related to housing fires rather than simply a piece of clothing catching fire, a range of tests relevant to fire safety of building materials was also taken into account.

The starting point in the process however was a very basic test, established to simply observe the differences in burning properties between different types of fabrics. Pieces of netting were exposed to a low temperature flame (a cigarette lighter) and the behavior monitored. Under the conditions of this test it was observed that samples of polyester and polyethylene netting caught alight (and in some cases continued to burn without auto extinguishing) whereas LifeNet (polypropylene) shrank away from the flame (melted) without catching alight. It was clear from this basic test that LifeNet behaved very differently from polyester and polyethylene nets.

Further investigation into testing protocols relating to flammability of fabrics revealed other types of flammability tests relevant to textile materials. The Norm Francaise classification (Safety against fire, building materials. Reaction to fire tests) requires three separate tests to be conducted:

1. NF P 92-503

   This is known as the electrical burner test used for flexible materials. Among the assessments within this test are the duration of ignition, whether or not burning drops fall from the fabric, and the spread of the afterglow.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Combustibility</th>
<th>Flammability</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>Non-combustible</td>
<td>Non-flammable</td>
</tr>
<tr>
<td>M1</td>
<td>Combustible</td>
<td>Flammable with difficulty</td>
</tr>
<tr>
<td>M2</td>
<td>Combustible</td>
<td>Average flammability</td>
</tr>
<tr>
<td>M3</td>
<td>Combustible</td>
<td>Easily flammable</td>
</tr>
<tr>
<td>M4</td>
<td>Combustible</td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>Non-classified</td>
<td>Non-classified</td>
</tr>
</tbody>
</table>

*Table 1*
2. NF P 92-504
   Test used for thermal melting materials. Dripping test. This test assesses whether or not ardent (burning) drops set alight to cotton.

3. NF P 92-505
   Flame persistence test and speed of the spread of flame. This test measures the duration (in seconds) of ignition, whether or not ardent (burning) drops fall and the speed of spread of any flame.

The Norm Francaise tests result in classification of textile materials according to Table 1.

From the classification reports, under the conditions of these tests, LifeNet (deltamethrin incorporated into polypropylene) was classified as M1 (non-flammable). This followed the observation that the maximum duration of ignition was only 1 second and there were no ardent drops.

The test reports also show that other net materials do not perform the same way. The results obtained for netting material based on polyethylene (with permethrin incorporated) would lead to a classification of M4 (easily flammable); this followed the observation that ignition lasted for 91 seconds and ardent drops were seen.

Similarly for netting material based on polyester (deltamethrin coated), the duration of ignition was 22 seconds, there were also ardent drops and the classification of M4 (easily flammable) was the result. These results are summarized in Table 2.

Due to the clear differentiation that can be established between textile materials based on a different set of tests, this poses the question – in the approval process for mosquito bednets, should the 16CFR Part1610 tests be supplemented or even replaced with a more robust range of tests that can clearly ascribe differences between materials?

Contact between nets and flames or sparks is a reasonably common problem, and can result in:

- Development of small holes in the net – providing weak points for easier tearing.
- Ignition of the net leading to development of larger holes in the net, allowing access of mosquitoes.
- Ignition of the net, causing skin burns. Certain types of net material catch alight more readily than others and those that generate drips of burning plastic present a risk of burns to persons sleeping under the net.
- More significant fires. Contact of lamps and/or candles with those net materials that burn readily could result in flames spreading to combustible materials adjacent to sleeping areas.

### CONCLUSION

The flammability of mosquito nets is a serious issue that has the potential to cause injury and damage. Current flammability testing requirements for mosquito nets can be considered inadequate to accurately define the flammability profile of a given material. Other test methodologies are available, and should be considered within the scope of testing required for mosquito nets. Using such tests polyester and polyethylene netting material caught alight and released burning drops, whereas polypropylene (LifeNet) melted away from the flame and was non-flammable.

<table>
<thead>
<tr>
<th>Net material</th>
<th>Duration of ignition (seconds)</th>
<th>Fall of Ardent drops</th>
<th>M Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polypropylene</td>
<td>1</td>
<td>No</td>
<td>M1 non-flammable</td>
</tr>
<tr>
<td>(LifeNet)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyethylene</td>
<td>91</td>
<td>Yes</td>
<td>M4 easily flammable</td>
</tr>
<tr>
<td>Polyester</td>
<td>22</td>
<td>Yes</td>
<td>M4 easily flammable</td>
</tr>
</tbody>
</table>

Table 2

**Article with references on the enclosed Public Health CD-ROM**
Since its foundation in 1980, CARE Deutschland, joined by Luxemburg in 2008 (CARE Deutschland-Luxemburg; CARE DL) has been supported by people who share its vision of a world without poverty, where everyone can live in peace with hope, tolerance, and social equality. Such supporting partners include the German government, the European Commission, the UN, and also companies, businesses, sponsors, and other non-profit organizations.

CARE DL focuses on worldwide emergency and disaster relief work as well as many other projects designed to reduce poverty. Supporting CARE International (see box) as a “partner in the fight against poverty”, CARE DL places a special focus on working alongside poor women because, equipped with the proper resources, women have the power to help whole families and entire communities escape poverty. Women are at the heart of CARE’s community-based efforts to improve basic education, prevent the spread of disease, increase access to clean water and sanitation, expand economic opportunity and protect natural resources. CARE also works to combat the root causes of poverty, CARE also works to improve agricultural practices, supports communities to prepare for a changing climate and works with marginalized groups such as the Roma in the Balkan region.

The organization aligns its work with emergency relief after natural disasters and in conflicts. To combat the root causes of poverty, CARE also works to improve agricultural practices, supports communities to prepare for a changing climate and works with marginalized groups such as the Roma in the Balkan region.

CARE Packages

After World War II when millions of people were homeless with hardly any food, clothes, or medical supplies, 22 American humanitarian organizations set up the “Cooperative for American Remittances to Europe” in Washington. With donations and support from hundreds of volunteers, CARE first sent food and basic supplies as CARE packages to war-torn Europe.

Almost 10 million CARE packages reached Germany between 1946 and 1960, helping people to overcome hunger and rebuild their lives. The first CARE packages...
were originally “10-in-1 food parcels”, US army rations intended to provide one meal for ten soldiers during the planned invasion of Japan. At ten dollars per package, senders initially had to specify who should receive them, but as donations flooded in, packages were distributed to all in need.

In March 1947 when these packages ran out, CARE started putting together its own packages containing a variety of food more suited to families. Assisted by generous donations from American companies, each box contained food with a total nutritional value of 40,000 kilocalories (see box) as well as clothes.

**Berlin Airlift**

When on June 24, 1948, the Soviet Union closed all supply lines to Berlin, the American allies set up the Berlin Airlift. This was a massive logistic project with aircrafts landing almost every minute to deliver essential supplies for the city of 2.5 million people who refused to give up hope. Planes chartered by CARE flew in more than 200,000 CARE packages of food, supplying 60% of the private aid for Berlin. At the beginning of 1949 the number of CARE packages reached 30,000 per month.

Altogether, between 1945 and 1960, 100 million CARE packages reached the European people in need. The CARE package had become a synonym for charity across all former enemy lines. By then CARE was sending aid to other countries in the world, and had extended relief to include health care programs. In the 1970s CARE responded to massive famines in Africa with emergency aid and relief programs. Nowadays CARE stands for “Cooperative for Assistance and Relief Everywhere”.

**Worldwide engagement**

For a post war generation in Germany CARE packages symbolized hope, compassion, and generosity. So when the worst was over in Europe, they wanted to support CARE programs helping people in developing countries. After the USA and Canada, the CARE organization was founded in Germany to help combat the growing divide between rich and poor. Many people in Germany were inspired by the CARE tradition to support those in need, regardless of religious, ethnic or political affiliation. Until today, CARE holds up these principles.

To more effectively combine their strengths and resources, all national CARE Organizations joined together to form CARE International in 1982. In recognition of its successful work and many years of experience, CARE has acquired an advisory status with the UN.


CARE International ensures high quality, professional and coordinated emergency relief and comprehensive programs to fight poverty. It expands the local and national work performed by member organizations such as CARE DL into a globally harmonized network.

Today, CARE is involved in many aspects of disaster relief and fighting poverty. In crisis zones, CARE supplies food, tents, blankets, clothes and essential survival items and medicines. It assembles mobile drinking water and toilets, teams of doctors and psychologists for medical help and trauma work, particularly for children and women. Initial aid is always linked to long-term development...
so people can rebuild their lives and protect themselves from future disasters.

Do no harm

It is always dangerous for aid workers in conflict zones. Experience shows that while they relieve suffering and save lives, they may also contribute to making the situation worse. The humanitarian mandate clearly states that aid organizations are to help those most in need, regardless of their role in the conflict. This sometimes puts aid workers in terrible dilemmas. In recognition of the fine line that humanitarian aid faces in conflict zones, a range of international aid organizations, including the UN, created the “Do no harm” concept in 1994. The central question is how can aid organizations carry out operations and encourage people to withdraw from the conflict to prepare the ground for peaceful solutions. The objective of “Do not harm” framework is to understand sources of tension, cohesion and capacities for maintaining peace within the context of a community and program.

This means that aid organizations in conflict zones carry a huge responsibility. The “Do no harm” project collects experiences from over 100 humanitarian organizations involved in crises and war zones worldwide. CARE is one of the founding partners of this project and maintains an active role. The project not only produces a handbook that is continually updated with training programs on how to proceed on location, but also organizes regular feedback workshops.

CARE projects

Providing access to financial services and trainings helps poor people, especially women, grow businesses, and ensuring education, particularly for girls, is a powerful tool for fighting poverty. Increasing access to clean safe water is essential for health, and CARE helps villages install drink water systems. Preventing food insecurity and hunger requires long-term solutions for productive cultivation, grain storage, and efficient management. These are just some of the projects where CARE DL works together with local communities to help them become more resilient to climate change, drought and other crises.

CONCLUSION

CARE DL works throughout Africa, Asia, Latin America, and Eastern Europe. In 2011, CARE International organized 1015 projects to help over 122 million people in 84 countries, and is one of the largest private aid organizations in the world. Emergency aid is carried out in cooperation with the UN and national or local organizations. Immediate responses are followed up with long-term development programs to help people make a new start.
Neglected tropical diseases: London declaration

At a meeting in London in January 2012, donors, endemic country governments, private sector leaders, and multilateral organizations made a unique commitment to launch the “London Declaration on Neglected Tropical Diseases”. They pledged to work together on a coordinated effort to control or eliminate 10 NTDs by 2020, in alignment with World Health Organization targets.

Bayer, as one of the 13 pharmaceutical companies represented at the meeting, joined in the largest coordinated effort so far to combat NTDs. Partners committed to sustaining or expanding existing drug donation programs to eradicate Guinea worm disease, and push forward progress for eliminating lymphatic filariasis, blinding trachoma, sleeping sickness and leprosy by 2020. Additional goals were control of soil-transmitted helminthes, schistosomiasis, river blindness, Chagas disease and visceral leishmaniasis.

Counting new and existing pledges, this means that companies will donate an average of 1.4 billion treatments per year to affected people. In addition partners agreed to share expertise and compounds to accelerate R&D to find new treatments. These commitments are to work in parallel with the R&D organization Drugs for Neglected Diseases initiative (DNDi) and WIPO Re:Search, a database of research compounds, knowledge, and expertise.

The pledges and the London Declaration follow the WHO strategy “Accelerating Work to Overcome the Global Impact of Neglected Tropical Diseases. A Roadmap for Implementation” (see page 14). The coordinated effort was developed by representatives from the pharmaceutical industry, the Bill & Melinda Gates Foundation and other partners over the previous year. “The efforts of WHO, researchers, partners, and the contributions of industry have changed the face of NTDs. These ancient diseases are now being brought to their knees with stunning speed,” said Margaret Chan, Director-General of the WHO.

Malaria movie: Mary and Martha

Director Richard Curtis’ latest film, Mary and Martha, a 90-minute drama for television (screened in Germany in April 2013), is about the preventable disease malaria. Partly autobiographical, the story derives from Curtis’ first visit to Ethiopia in 1985, which inspired him and Lenny Henry to found Comic Relief.

The story revolves around two women, Mary, a wealthy American (Oscar-winning Hilary Swank) and British working class Martha (Bafta-winner Brenda Blethyn). These two women from completely different worlds are brought together in Mozambique by the shared tragedy of losing their sons to malaria. They then set about campaigning to raise awareness about the disease.

Mary takes her young son to Africa where he catches malaria and dies. Martha goes to work in a Mozambique orphanage where her son had died when working as a volunteer. The two very different women form a lasting friendship full of humor and courage in their effort to help rid the world of malaria.

The campaigning is mostly left to Mary, the American, but this was intentional, says Curtis “because the UK government is doing so well.” The UK is the first G20 country this year to honor its commitment to spend 0.7% of its gross National Income on international development.
Reorganizing the Global Fund: Investing for impact

2012 was a year of comprehensive reorganization and self-assessment for the Global Fund to Fight AIDS, Tuberculosis and Malaria. Important changes were made to strengthen its organizational structure and governance operations, based on the internal reforms set out in the Consolidated Transformation Plan (CTP). To implement the CTP the Fund created three streamlined committees: Finance and Operational Performance (FOPC), Strategy, Investment and Impact (SIIC), and Audit and Ethics (AEC). Each committee includes a Private Sector Delegation (PSD) member from companies with a strong interest in the Global Fund. PSD activities in turn are financially supported and governed by the PSD Advisory Group, a small group of 18 companies (incl. Bayer since 2012). Importantly, the Advisory Group provides technical expertise and corporate sector know-how in areas such as grant management, financial controls, risk mitigation, resource mobilization, and attracting private sector engagement and contributions to the Global Fund.

The Global Fund’s new strategy for 2012-2016 is to invest more strategically and ensure that the highest impact interventions focus on areas of greatest need. Over the next 5 years the aim is to save 10 million lives and prevent 140-180 million new cases of HIV/AIDS, TB and malaria. This will necessitate attracting, and distributing better grants with increased impact to make a sustainable and significant contribution to reducing these diseases, and hence poverty as one of the MDGs.

Sanofi Pasteur: Dengue vaccine demonstrates proof of efficacy

The results of a study conducted in Thailand, published in the Lancet, September 2012, showed protection against three out of four dengue virus serotypes as well as an excellent vaccine safety profile. The vaccine efficacy was 61.2% against dengue virus type 1, 81.9% against type 3, and 90% against type 4. One of the dengue virus types (serotype 2) eluded the vaccine, and analyses are ongoing to understand the lack of protection for serotype 2 in the particular epidemiological context of Thailand. The study was conducted in 4,002 children aged 4 to 11 years, in partnership with the Mahidol University under the patronage of the Thai Ministry of Public Health in Muang district of the Ratchaburi Province. Sanofi Pasteur’s dengue vaccine candidate is a live, attenuated vaccine. The vaccination schedule is three doses given six months apart (at 0, 6 and 12 months). Currently, large-scale phase III clinical studies of Sanofi Pasteur’s dengue vaccine candidate are underway with 31,000 children and adolescents in ten countries in Asia and Latin America. These studies will generate important additional data in a broader population and in a variety of epidemiological settings to define the best conditions to set up vaccination programs in order to protect people at risk of dengue.

Source

Dengue fever

As a pandemic disease, dengue fever is quite recent – and also a cautionary tale about a disease developing new niches and pathogenicity as a result of human activities. This is compounded by the fact that the dengue virus diversified into four types, and extended its range of transmission to mosquitoes that thrive in urban areas. Both factors play critical roles in escalating infections into lethal dengue hemorrhagic fever.

Although early Chinese writings described a dengue-like disease in 992, molecular evolution studies suggest that the dengue virus first entered a sustained human-mosquito-human cycle between 320 and 125 years ago. These viruses, belonging to the family Flaviviridae, genus Flavivirus, apparently diversified into four serotypes in monkeys in South East Asia, the only location where all four types have been identified in forest cycles.

Dengue probably spread to the Americas in the late 1600s, although at this time just as a flu-like fever, causing extreme aches and pains. The original vector at its source, the tiger mosquito Aedes albopictus – with its striped legs – prefers dense vegetation. So at first urbanization reduced dengue. Then Aedes aegypti arrived in the New World from Africa, probably with slave trade ships and colonists. As it spread worldwide, it created a new niche of transmission. The Aedes aegypti mosquito is not only highly adapted to living in areas of human habitation, it also became the major transmitting vector.

In a first clinical description in 1780, during the American Revolution, the army surgeon Benjamin Rush called the disease break-bone fever – although this may have included chikungunya infections. In 1906, the young US Army physicians Percy Ashburn and Charles Craig, working in the Philippines, managed to transfer the disease using filtered blood serum from dengue patients. This proved that dengue fever was not caused by bacteria or parasites, but by a virus, the second human viral pathogen discovered after the yellow fever virus described by Reed in 1901. Although lending support to work of Graham (1903) that dengue is transmitted by mosquitoes, this was not proven conclusively until the results obtained by Siler, Hall and Hitchens in 1924: They demonstrated direct patient-to-volunteer infection via the mosquito vector Aedes aegypti.

**Emergence of dengue hemorrhagic fever**

A massive campaign in the Americas during construction of the Panama Canal was started by the French in 1880, but soon turned into a battle against bankruptcy and mosquitoes. By the time it was completed by the USA in 1914 thousands of workers had died from malaria, yellow fever, and dengue.
**AEDES AEGYPTI**, the major vector for dengue diseases, breeds near human habitation in man-made domestic water storage systems, and discarded plastic containers or automobile tires that collect rainwater. The only methods of controlling dengue fever are applying larvicides, e.g. organophosphates, to interrupt vector breeding, space sprays using pyrethroids and thermal fogging to kill adult mosquitoes. Long-lasting insecticide-treated materials can help provide protection.

the Panama Canal, primarily to combat yellow fever, also eradicated dengue since both share the same vector mosquito. But *Aedes aegypti* was never exterminated in other parts of the tropics and continued to spread eastwards. In the 1950s dengue fever made a comeback in the Philippines and Thailand and this time urban populations were hit hard, and for the first time with a much more virulent form. This marked the start of an epidemic in Manila, but it was still a mystery as to why dengue fever was suddenly progressing into a severe and fatal disease. It was not until the 1970s that previous infection with a different dengue virus serotype, or the presence of maternally derived antibodies was recognized as a risk factor for developing DHF. How immunity to, or previous infection by one serotype can trigger the severe DHF shock response to a second infection is still not fully understood. But it does mean that developing a safe and effective vaccine against dengue is difficult – it must protect against all 4 virus serotypes simultaneously. Otherwise it simply increases the risk of fatal infection.

Dengue research during World War II discovered dengue viruses 1 and 2. Serotypes 3 and 4 were identified in 1956 during hyperendemicity (co-circulation of multiple virus serotypes now acquired by *Aedes aegypti*) and the emergence of dengue hemorrhagic fever (DHF). This more lethal form started killing thousands of people in Asia.

Other methods of protection and control are complicated by the fact that many methods of adult mosquito control are ineffective against the domesticated *Aedes aegypti*. This puts the focus on larval control. But despite vector control strategies, dengue has been resurging over the last 30 years, especially in Central and South America. The WHO estimates that 2.5 to 3 billion people are at risk, with 50 to 100 million new dengue infections per year. About 500,000 cases of DHF, mostly children, are treated in hospitals each year, and thousands of these patients die.*

**PERCY ASHURN** (left) and **CHARLES CRAIG** studied dengue fever through a series of carefully designed experiments with volunteers from the US army during an epidemic in the Philippines in 1906.

**A modern pandemic**

Dengue fever and DHF are considered the most widespread reemerging diseases, a pandemic resulting from modern demographics and life-styles. Population explosion, unplanned urban expansion, plastic container pollution, and rapid transportation of large numbers of people have resulted in dramatic geographical spread of the disease. Global climate change is likely to be another key issue affecting future epidemics, possibly putting more people at risk of multiple infections. The future of dengue still depends on human activities.

* see statistics on pages 5, 9, 15
We wish you a pleasant and informative read.

DENGUE AND CHIKUNGUNYA

The tiger mosquito, *Aedes albopictus*, a vector of chikungunya and dengue viruses, and its cousin *Aedes aegypti*, the principle vector of dengue fever, have both evolved life cycles closely linked to human habitation. Females like to lay eggs in domestic water and sanitation systems, and any collected water such as in used tires and discarded water containers.
Link List

With reference to the topics in this issue of Public Health Journal we include a summary of the main Internet links, where you can find further information, the latest reports and statements.

Bayer Vector Control
www.vectorcontrol.bayer.com

Bill & Melinda Gates Foundation
www.gatesfoundation.org

CARE Deutschland-Luxemburg
www.care.de

CARE International
www.careinternational.org

CDC (Centers for Disease Control and Prevention): Dengue Fever & Dengue Hemorrhagic Fever
www.nc.cdc.gov/.../yellowBookCh4-DengueFever

DFID (Department for International Development)
www.dfid.gov.uk

ECDC (European Centre for Disease Prevention and Control)
www.ecdc.europa.eu

Fast-track Diagnostics (FTD)
www.fast-trackdiagnostics.com

Global Fund to Fight AIDS, Tuberculosis and Malaria
www.theglobalfund.org

Liverpool School of Tropical Medicine
www.liv.ac.uk/lstm

London School of Hygiene and Tropical Medicine
www.lshtm.ac.uk

Mauritius: Ministry of Health and Quality of Life
http://health.gov.mu

Oxitec
www.oxitec.com

Pakistan: Inter Provincial Coordination Division, Government of Pakistan
www.ipc.gov.pk

Sanofi Pasteur
www.sanofipasteur.com

WHO (Neglected tropical diseases)
www.who.int/neglected_diseases

World Malaria Report

Events

6th International Congress of SOVE (Society for Vector Ecology)
September 22-27, 2013
Palm Springs (La Quinta), California, USA
www.sove.org

3rd International Conference on Dengue and DHF
October 9-11, 2013
Bangkok, Thailand
www.dengue2013bangkok.com

13th International Congress of Parasitology
August 10-15, 2014
Mexico City, Mexico
www.icopa2014.com

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PUBLIC HEALTH JOURNAL: No. 24 on CD-ROM

As a special service for readers of Public Health Journal we include a CD-ROM (see inside back cover). Not only does it contain every page of the complete issue in pdf format, but also the individual articles. Some feature additional information.

DENGUE AND CHIKUNGUNYA
The tiger mosquito, Aedes albopictus, a vector of chikungunya and dengue viruses, and its cousin Aedes aegypti, the principle vector of dengue fever, have both evolved life cycles closely linked to human habitation. Females like to lay eggs in domestic water and sanitation systems, and any collected water such as in used tires and discarded water containers.

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